The International Pharmacopoeia

Fourth edition

Volume 2

World Health Organization

Geneva
2006

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The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization's priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO's Member countries and the collaboration of world leaders in public health and the biomedical sciences.

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective – the attainment by all people of the highest possible level of health.

The International Pharmacopoeia

FOURTH EDITION

Pharmacopoea Internationalis
Editio Quarta

Volume 1

World Health Organization
Geneva
2006

WHO Library Cataloguing-in-Publication Data

The International pharmacopoeia = Pharmacopoea internationalis. – 4th ed.

2 v.

1.Pharmacopoeias 2.Pharmaceutical preparations – standards 3.Pharmaceutical preparations – analysis 4.Dosage forms – standards I.World Health Organization.

ISBN 92 4 156301 X (LC/NLM classification: QV 738.1)

Printed in Singapore

Contents

Preface

The International Pharmacopoeia[1] (Ph. Int.) comprises a collection of recommended procedures for analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms that is intended to serve as source material for reference or adaptation by any WHO Member State wishing to establish pharmaceutical requirements. The pharmacopoeia, or any part of it, shall have legal status, whenever a national or regional authority expressly introduces it into appropriate legislation. Further explanation of the role of *The International Pharmacopoeia* is provided in the paragraphs entitled "Scope and function" at the end of this Preface and a summary of its historic development is provided under History.

This is the fourth edition of *The International Pharmacopoeia*. It comprises two volumes; the General Notices and monographs for pharmaceutical substances (A to O) are to be found in Volume I and the remaining monographs for pharmaceutical substances together with those for dosage forms and radiopharmaceutical preparations, the methods of analysis and the reagent sections are to be found in Volume 2. The main reason for publishing a new edition at this time is to consolidate the texts of the five separate volumes of the third edition and to include those new monographs for antiretroviral substances that were adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2004.

In preparing this consolidated edition, the opportunity has been taken to improve certain aspects of the layout and format of the publication. In this edition all the monograph texts have been brought together in one main section and the method texts in another. Each of these major sections has been divided into appropriate sub-sections and the method texts have been numbered for ease of cross-reference.

General Notices During preparation of this edition a review was carried out of the General Notices. Certain additions and amendments have been made in order to clarify the interpretation of the Pharmacopoeia and to facilitate application of the requirements by the user. New General Notices have been added for Definition, Manufacture and Impurities. The new notice on Definition serves to define dosage forms as being made with active ingredients of pharmacopoeial quality and to clarify the mandatory status of certain state-

[1] Published in accordance with World Health Assembly resolution WHA3.10, *WHO Handbook of Resolutions and Decisions*, Vol. 1, 1977, p. 127.

ments in monographs. The new notice on Manufacture governs the interpretation of statements included under this heading in monographs such as the general monographs for dosage forms, the monographs for the different grades of water and certain other individual monographs, for example Bleomycin hydrochloride and Desferoxamine mesilate. The need for a notice on Impurities arose from the inclusion of information at the end of certain of the new monographs for antiretoviral substances. Such lists of known and potential impurities that have been shown to be controlled by the tests in a monograph are likely to be included more widely in future.

Notices that have been significantly revised include those for Labelling, General Requirements and Tests and Assays. General methods of analysis that are commonly used in carrying out the tests and assays included in the monographs of *The International Pharmacopoeia* are described in the section on Methods of analysis. In some cases a specific cross-reference to the method required is provided within the monograph text. Examples include references to Spectrometry in the infrared region, High performance liquid chromatography (HPLC) and Limit test for heavy metals. In other cases, where the relevant method can be inferred from the title of the test, no explicit cross-reference is given. Examples include tests for Specific optical rotation, Sulfated ash, Loss on drying and pH value. A statement has been added to the General notice on General requirements to assist in the correct interpretation of the monograph requirements, especially in those cases where there is no cross-reference. It emphasizes that whether or not a specific cross-reference is included, the requirements of the monographs of *The International Pharmacopoeia* are to be interpreted in relation to the relevant method of analysis.

New monographs New monographs are included for the following antiretroviral substances: Didanosine, Indinavir sulfate, Nelfinavir mesilate, Nevirapine, Ritonavir, Saquinavir and Saquinavir mesilate. These monographs have been developed as part of the WHO strategy to make quality antiretroviral medicines more widely available to HIV-positive patients. Such specifications support the joint UNICEF-WHO-UN Prequalification project[1], managed by WHO. These new monographs provide an element of choice in relation to test methods used for identification and, where possible, a titration method for assay, in line with established policy. However, in order to provide adequate control of impurities, it has been found necessary to place reliance on HPLC.

Revision The monograph for Oral rehydration salts has been revised to conform to the modified formula as published in the 13th Model List of Essential Medicines[2] and in the Model Formulary 2004[3]. The revised formula has a reduced sodium chloride and glucose content providing a solution with a reduced osmolarity of 245 mOsm/l. Due to the improved effectiveness of the reduced osmolarity ORS solution, especially for children with acute, non-

[1] WHO Technical Report Series, No. 933, 2005, p. 13 and, for current project information, consult the prequalification website (http://mednet3.who.int/prequal).
[2] WHO Technical Report Series, No. 920, 2003.
[3] WHO Model Formulary, Geneva, World Health Organization. 2004.

cholera diarrhoea, WHO and UNICEF now recommend that countries use and manufacture this formulation in place of the previously recommended ORS, that is, the one published in the third edition of *The International Pharmacopoeia*, which had a total osmolarity of 311 mOsm/l.

The general monograph for Parenteral Preparations has been amended to make reference to the test for bacterial endotoxins as the preferred method for ensuring the quality of preparations with respect to pyrogens. Individual monographs no longer include recommended storage temperatures where these are covered by the normal storage conditions as defined in the General Notices.

Method texts that have been updated include, for example, the text on High performance liquid chromatography (HPLC). This has been revised to clarify certain technical terms and to add advice on adjustment of chromatographic conditions as recommended by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

CD-ROM This fourth edition is published simultaneously both in print and on CD-ROM. This provides the user of *The International Pharmacopoeia* with a choice of form in which to consult the publication depending on the circumstances and the type of enquiry. The response from users of the CD-ROM of the third edition, published in 2004, has demonstrated the usefulness of making the publication available electronically. The simplified structure of the fourth edition and the improved functionality of the CD-ROM will facilitate both reading and especially searching the text.

Future Work is in progress on the preparation of new monographs for antiretroviral substances, for the associated dosage forms and for a number of fixed-dose combination products for the treatment of HIV/AIDS and of tuberculosis. Revision of monographs also continues in order to improve specifications, for example, by providing better means of impurity control or by the addition of a dissolution test. It is intended to make additions and revisions to the fourth edition available, at appropriate intervals, by means of printed Supplements and updated CD-ROMs. Meanwhile, attention is drawn to the WHO Medicines website (htpp://www.who.int/medicines), where finalized texts of monographs adopted by the Expert Committee are provided for information together with much other detailed information of relevance to all those responsible for the quality assurance of medicines.

Scope and function

The activities related to *The International Pharmacopoeia* are an essential element in the overall quality control and assurance of pharmaceuticals contributing to the safety and efficacy of medicines.[1] The selection of monographs for inclusion in *The International Pharmacopoeia* recognizes the needs of specific disease programmes and the essential medicines nominated under these programmes;

[1] WHO Technical Report Series, No. 908, 2003, Annex 2.

it is based primarily on those substances included in the current *WHO Model List of Essential Medicines*.[1] The work on *The International Pharmacopoeia* is carried out in collaboration with members of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations as well as with specialists from regulatory authorities, from industry and from other institutions (see under Acknowledgements). Clearly defined steps are followed in the development of new monographs.[2]

It is emphasized that pharmacopoeial specifications represent only one element of the quality assurance of medicines. Pharmaceutical substances and dosage forms for human use, as described in a monograph of *The International Pharmacopoeia*, should be manufactured according to the current requirements of Good Manufacturing Practices (GMP)[3] The processes, premises, equipment, and installations should also comply with the provisions of the product licence or marketing authorization, relevant regulations and, in the case of products destined for export, with any binding international norms that would affect their entry onto the market. In many cases this compliance cannot be verified by analysing a sample of the final product against a pharmacopoeial monograph. The national, regional or other competent authority will need to ensure that all relevant provisions have been met by any means at its disposal, including use of appropriate certificates, inspection of the manufacturing sites or testing of samples beyond specifications.

It should be understood that a distinction exists between pharmacopoeial standards and manufacturers' release specifications. Pharmacopoeial standards are publicly-available compliance specifications and provide the means for an independent check of the quality of a product at any time during its shelf-life. Although release specifications must be compatible with pharmacopoeial specifications, they may differ in several respects. In order to ensure compliance with the pharmacopoeia, the manufacturers' specifications may need to be more exacting than corresponding pharmacopoeial requirements. The manufacturer is entitled to use other analytical methods for routine testing and, moreover, he may assure himself that the requirements of the pharmacopoeia will be met by means other than routinely performing all of the tests in the monograph. For example, on occasions, in-process controls and manufacturing process validation studies may have already provided the necessary assurances with respect to certain aspects of the monograph.

The requirements of the monographs are not framed to detect all possible impurities. The present tests are designed to determine impurities on which attention should be focused, to fix the limits of those that are tolerable to a certain extent, and to indicate methods for ensuring the absence of those that are undesirable. It is, therefore, not to be presumed that an impurity can be tolerated because it has not been precluded by the prescribed tests. In some purity

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[1] WHO Technical Report Series, No. 933, 2005.
[2] WHO Technical Report Series, No. 929, 2005. p. 5.
[3] For the current WHO recommendations, consult the WHO Medicines website (htpp://www.who.int/medicines).

tests, limits are indicated additionally in brackets in percentage terms: as stated in the General Notices, such limits are given for information only.

Pharmaceutical preparations (dosage forms) that are produced on a large scale and that will be stored before use should undergo testing to show physical and chemical stability during storage over the claimed shelf-life.[1]

The degree of protection provided by pharmacopoeial standards will depend not only on their technical content but also to a great extent on how they are used. The specified tolerances and limits allow for the inherent variations that occur during production and packaging, as well as for subsequent degradation within normal handling and storage conditions and for any acceptable variance of analytical results.

* * *

When pharmacopoeial standards are used to establish the compliance of products with regulatory requirements, the following principles apply:

- **The interpretation of a monograph must be in accordance with all general requirements and testing methods, texts, or notices pertaining to it as found in this edition.**
- **No further tolerances are to be applied to the limits prescribed.**
- **A product is not of pharmacopoeial quality unless it complies with all the requirements stated.**

* * *

[1] *Quality assurance of pharmaceuticals: a compendium of guidelines and related materials.* Volume 1. Geneva, World Health Organization, 1997. For the current WHO recommendations, consult the WHO Medicines website (htpp://www.who.int/medicines).

History

The history of *The International Pharmacopoeia* dates back to 1874 when the need to standardize terminology and to specify dosages and composition of drugs led to attempts to produce an international pharmacopoeial compendium. The first conference, called by the Belgian Government and held in Brussels in 1902, resulted in the Agreement for the Unification of the Formulae of Potent Drugs, which was ratified in 1906 by 19 countries. The outcome considerably influenced the subsequent publication of national pharmacopoeias.

A second agreement, the Brussels Agreement, was drawn up in 1925 and ratified in 1929. This 41-article agreement stipulated that the League of Nations would be responsible for the administrative work to produce a unified pharmacopoeia, and a permanent secretariat of an international organization would coordinate the work of national pharmacopoeial commissions. General principles for the preparation of galenicals, maximal doses, nomenclature, and biological testing of arsenobenzones were included in the articles of this agreement, as was a table of dosage strengths and descriptions for 77 drug substances and preparations.

In response to repeated calls from pharmaceutical experts in various countries that the Brussels Agreement be revised and extended to cover an international pharmacopoeia, the Health Organization of the League of Nations set up a Technical Commission of Pharmacopoeial Experts in 1937. This first committee comprised seven experts from Belgium, Denmark, France, Netherlands, Switzerland, the United Kingdom (Chairman), and the United States of America.

In 1947 the Interim Commission of WHO took over the work on pharmacopoeias previously undertaken by the Health Organization of the League of Nations, and set up an Expert Committee on the Unification of Pharmacopoeias to continue the work of the League's Technical Commission. The aim of the Expert Committee was to produce a draft international agreement for the unification of pharmacopoeias, modifying and extending the existing Agreement for the Unification of the Formulae of Potent Drugs.

In 1948 the First World Health Assembly approved the establishment of the Expert Committee by the Interim Commission. In 1951 this became the Expert Committee on the International Pharmacopoeia; and subsequently, in 1959, the Expert Committee on Specifications for Pharmaceutical Preparations. The panel has always been named the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations.

Article 2 of the WHO Constitution states that one of the functions of the Organization is "to develop, establish and promote international standards with respect to food, biological, pharmaceutical and similar products". *The International Pharmacopoeia* falls clearly into this category. In this context also the Third World Health Assembly in 1950 adopted a resolution to create the International Nonproprietary Names (INN) Programme in order to identify pharmaceutical substances unambiguously on a worldwide basis and to provide a single nonproprietary name to be used in monographs.

First edition

The Third World Health Assembly, held in May 1950, formally approved the publication of the *Pharmacopoea Internationalis* and recommended, in accordance with Article 23 of the WHO Constitution, "the eventual inclusion of its provisions by the authorities responsible for the pharmacopoeias". It was thus recommended that the *Pharmacopoea Internationalis* was not intended to be a legal pharmacopoeia in any country unless adopted by the pharmacopoeial authority of that country. From that moment the World Health Organization constituted the Permanent International Pharmacopoeia Secretariat.

The first edition, published with the aim of creating a worldwide, unified pharmacopoeia, relied on collaboration with national pharmacopoeia commissions for its preparation. It was published in two volumes (1951 and 1955) and a supplement (1959) in English, French and Spanish, and was also translated into German and Japanese. Altogether, it included 344 monographs on drug substances, 183 monographs on dosage forms (capsules, injections, tablets and tinctures) and 84 tests, methods, and general requirements.

A large number of national pharmacopoeias and official lists were examined and assistance was also obtained from the International Pharmaceutical Federation (FIP) to determine the selection of substances and products to be described in the pharmacopoeia. Latin was chosen for the monograph titles because of its distinction as an international language. Experts collaborated with the WHO Expert Committee on Biological Standardization with regard to biological products, and with those working in specific divisions, e.g. malaria, maternal and child health, mental health, and venereal diseases, to help collate the required information.

Second edition

The second edition was published in 1967 as *Specifications for the Quality Control of Pharmaceutical Preparations*, with a subtitle classifying it as the second edition of *The International Pharmacopoeia*.

Owing to the development of new analytical techniques such as infrared spectroscopy, chromatography (column, paper and thin-layer), non-aqueous titration, and radioactivity, the second edition incorporated numerous alterations and constituted a revision of the first edition.

The selection of monographs and appendices was based largely on the availability, at the time of preparation, of specifications intended for publication in

national pharmacopoeias and in other volumes of specifications for pharmaceutical quality control. Specifications for 162 pharmaceutical preparations not included in the first edition were introduced in the second edition, while 114 monographs were deleted, based on feedback from the first edition. New analytical methods were also added. The specifications and methods in the monographs were tested in a number of national pharmacopoeial and pharmaceutical quality control laboratories, in pharmaceutical manufacturers' laboratories, and at various pharmacopoeial institutes.

Special thanks were expressed to the authorities of the British Pharmacopoeia and the United States Pharmacopeia.

Third edition

In 1975 the purpose of *The International Pharmacopoeia* was reconsidered. It was decided that the publication should focus more on the needs of developing countries and recommend only simple, classical chemical techniques that had been shown to be sound. Priority would be given to drugs that were widely used throughout the world, with emphasis on the therapeutic value of these drugs. High priority would be accorded to drugs important to WHO health programmes, and to those likely to contain impurities arising from degradation or due to difficulties in their manufacture. Wherever possible, classical procedures would be used in the analytical methods so that the pharmacopoeia could be applied without the need for expensive equipment. Where a sophisticated analytical method was suggested, an alternative, less complex method would also be proposed.

Since 1979, the drugs appearing in *The International Pharmacopoeia* have been selected from the list of essential drugs based on the first report of the WHO Expert Committee on the Selection of Essential Drugs. Specifications are provided in the monographs for the identification, purity, and content of the essential drugs appearing in the WHO Model List of Essential Drugs, and their updates.

The Third edition eventually consisted of five volumes: Volume 1 contained general methods of analysis; Volumes 2 and 3, quality specifications for the majority of essential drug substances in the WHO Model List of Essential Drugs and Volume 4, information on tests, methods, and general requirements and quality specifications for pharmaceutical substances, excipients, and dosage forms. Volume 5, the final volume, contained tests and general requirements for dosage forms and quality specifications for pharmaceutical substances and tablets, which practically completed the list of monographs for active pharmaceutical substances, and a section on antimalarial drug substances and their most widely used dosage forms.

Fourth edition

The fourth edition of *The International Pharmacopoeia* comprises two volumes published together; Volume I contains the General Notices and many of the monographs for pharmaceutical substances and Volume 2 contains the remain-

ing monographs for pharmaceutical substances together with those for dosage forms and radiopharmaceutical preparations, the methods of analysis and the reagents section and index. This edition consolidates and updates the texts of the five separate volumes of the third edition and includes new monographs for antiretroviral substances. The fourth edition is published simultaneously both in print and on CD-ROM.

Acknowledgements

The specifications included in the fourth edition were developed in collaboration with members of the WHO Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations, other specialists, and the WHO Collaborating Centres on quality control and quality assurance.

Thanks are also due to the Controller of Her Majesty's Stationery Office, the European Pharmacopoeia Commission and the United States Pharmacopeial Convention, Inc. for providing valuable background material.

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Furthermore, comments were obtained from the International Federation of Pharmaceutical Manufacturers Associations, Geneva, Switzerland; the World Self-Medication Industry, Ferney-Voltaire, France; Pharmacopoeial Commissions, national institutes for quality control of drugs, and drug research laboratories, and from the following WHO Collaborating Centres: WHO Collaborating Centre for Drug Quality Assurance, Therapeutic Goods Administration Laboratories, Department of Community Services and Health, Woden, ACT, Australia; WHO Collaborating Centre for Drug Quality Assurance, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China; WHO Collaborating Centre for Biopharmaceutical Aspects of Drug Quality Control, Laboratory of Biopharmacy, Faculty of Pharmacy, University of Clermont-Ferrand, Clermont-Ferrand, France; the former WHO Collaborating Centre for Stability Studies of Drugs, Regional University Hospital, Nantes, France; WHO Collaborating Centre for Drug Information and Quality Assurance, Budapest, Hungary; WHO Collaborating Centre for Quality Assurance of Essential Drugs, Central Drugs Laboratory, Calcutta, India; the former WHO Collaborating Centre for Quality Assurance of Essential Drugs, Directorate General of Drug and Food Control, Ministry of Health, Jakarta, Indonesia; the former WHO Collaborating Centre for Drug Quality Control, State Research Institute for the Standardization and

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The World Health Organization takes this opportunity to express its gratitude to all those people and institutions involved in the preparation of this 4th edition and its special thanks to Professor I. Addae-Mensah, Professor J. Hoogmartens, and Miss M.L. Rabouhans who also served as Chairpersons, and to Mrs K. Bremer, Dr J.A. Molzon, Dr R. Pandjaitan, Professor Jin Shaohong, Ms Metta Treebamroong and Professor Yang Zhong-Yuan who also served as Vice-Chairpersons of the WHO Expert Committee on Specifications for Pharmaceutical Preparations from 1995 to 2005. Members of the WHO Secretariat involved in the elaboration of the publication were Dr S. Kopp (Secretary to the above Expert Committee), Mrs W. Bonny, Mrs A.N. Lo Conte, Miss M.L. Rabouhans, Miss M. Schmid and Dr P. Vanbel.

General Notices

General Notices: Contents

Monograph nomenclature

The main monograph title is given in Latin and English. A singular Latin form of the recommended or proposed International Nonproprietary Name (INN) is applied, unless otherwise indicated.

All names of substances, with certain traditional exceptions, are treated as second declension neuter substantives (e.g. Ethosuximidum).

The method of nomenclature adopted for salts is the traditional one of placing the name of the acid component in the nominative case (either second declension neuter or third declension masculine) and the other component in the genitive case (e.g. Codeini phosphas). With compounds that are not derived from true acids, both components of the title are placed in the nominative case, treating the main component as a neuter substantive and using an adjectival form of the complementary component in agreement with this substantive (e.g. Cloxacillinum with "natricus" as the adjectival form of Natrium, thus Cloxacillinum natricum).

Dosage forms are chosen by placing the Latin name of the dosage form in the nominative case and the active ingredient in the genitive case, (e.g. Ampicillini capsulae, Ephedrini sulfatis injectio). Whenever the dosage form is intended for reconstitution, this is indicated by using the word "ad" and the appropriate application in the fourth declension (e.g. Ampicillini natrici pulvis ad injectionem).

Chemical formula and relative molecular mass

When the chemical composition of a pharmacopoeial substance is known or generally accepted, the empirical chemical formula and the relative molecular mass are given. For organic substances, the graphic formula, when known or generally accepted, is also given. These formulae and relative molecular masses are given at the beginning of the monographs and are those of the chemically pure substances; they are not to be regarded as an indication of the purity of the substance under test. Elsewhere, in statements of specifications of purity and strength, and in descriptions of processes for assay, it is evident from the context that the formulae denote the pure chemical substances.

Chemical names

The chemical names are given in accordance with the rules laid down by the International Union of Pure and Applied Chemistry (IUPAC). In many cases, when equally acceptable alternative names may be construed under these rules, more than one systematic name is given. Such alternative names are given especially when changes in the interpretation of IUPAC rules occurring in recent years have led to substantial modifications of the chemical names used for the substance. The recognition of substances is further facilitated by a registry number established by the Chemical Abstracts Service of the American Chemical Society (CAS No.).

Other names

Commonly used synonyms are given to aid identification of the article in question.

Definition

Statements given under the heading Definition or immediately after the heading Requirements constitute the official definition of the substance or dosage form that is the subject of the monograph. They constitute instructions or requirements with which the article must comply.

A dosage form that is the subject of a specific monograph must be prepared using an active ingredient that complies with the corresponding substance monograph. For example, the active ingredient used in preparing Artesunate tablets must comply with the monograph for Artesunate. Similarly, where an excipient for which there is a pharmacopoeial monograph is used in preparing such a dosage form, the excipient must comply with that monograph. For example, if lactose is used in preparing Artesunate tablets, it must comply with the monograph for Lactose.

Manufacture

The manufacturing facilities and the manufacturing process for a substance or dosage form that is the subject of a monograph in the International Pharmacopoeia must meet the current WHO requirements of Good Manufacturing Practice[1]. Where applicable, substances must be manufactured in accordance with the WHO "Recommendations on Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products" reproduced in the section Supplementary Information.

Statements under the heading manufacture draw attention to particular aspects of the manufacturing process but are not necessarily comprehensive. They may be in the form of mandatory instructions to manufacturers or, where clear from the form of wording used, they may provide guidance. In the general monographs for dosage forms, information is given that is intended to provide broad guidelines concerning the main steps to be followed during production, indicating those that are most important.

Description

Statements given under this heading are not to be interpreted in a strict sense and are not to be regarded as analytical requirements.

Solubility

Unless otherwise indicated, the approximate solubility of a substance is evaluated at 20 °C. The expression "part" describes the number of millilitres (ml)

[1] For the current WHO recommendations, consult the WHO Medicines website (htpp://www.who.int/medicines).

of solvent represented by the stated number of parts in which 1 gram (g) of solid is soluble.

Very soluble	Less than		1 part
Freely soluble	From	1 to	10 parts
Soluble	From	10 to	30 parts
Sparingly soluble	From	30 to	100 parts
Slightly soluble	From	100 to	1000 parts
Very slightly soluble	From	1000 to	10000 parts
Practically insoluble	More than		10000 parts

Category

The statements given for information are intended to indicate the principal pharmacological action and therapeutic use or, for excipents, the main pharmaceutical use. It should not be assumed that the substance has no other action or use. The statements are in no way intended to be binding.

Storage

Substances, dosage forms, and other materials must be stored under specified conditions in order to avoid contamination and deterioration.

(a) Containers

The container and its closure must not interact physically or chemically with the substance within in any way that would alter its quality. The following terms include general requirements for the permeability of containers:

Well-closed containers must protect the contents from extraneous matter or from loss of the substance under normal conditions of handling, shipment, or storage.

Tightly closed containers must protect the contents from extraneous matter, from loss of the substance, and from efflorescence, deliquescence, or evaporation under normal conditions of handling, shipment, or storage. If the container is intended to be opened on several occasions, it must be designed to be airtight after reclosure.

Hermetically closed containers must protect the contents from extraneous matter and from loss of the substance, and be impervious to air or any other gas under normal conditions of handling, shipment, or storage.

In addition, a *tamper-evident container* is one that is fitted with a device that reveals clearly whether it has ever been opened.

(b) Protection from light

Substances and dosage forms requiring protection from light should be maintained in a light-resistant container that – either by reason of the inherent properties of the material of which the container is composed, or because a special coating has been applied to the container – shields the contents from the effects

of light. Alternatively, the container may be placed inside a suitable light-resistant (opaque) covering and/ or stored in a dark place.

(c) Temperature
Where storage at temperatures other than room temperature (15 to 25 °C or, depending on the climatic conditions, up to 30 °C) is recommended, this is stated in the monograph. Such substances and dosage forms should be labelled accordingly.

Stability information
For substances and dosage forms that deteriorate easily under adverse storage conditions (such as occur in tropical climates), a warning should be given indicating that degradation is likely to occur in a humid atmosphere and that decomposition is faster at elevated temperatures. In such cases this warning is given in the section "Additional information".

Labelling
Official national and regional labelling requirements should be met.

When the term "label" is used in the International pharmacopoeia, the labeling statements may appear on the container, the package or a leaflet accompanying the package, as decided by the relevant authority.

For dosage forms, the label should state whether an antimicrobial preservative has been added, its name and concentration, as well as those of other substances such as buffers and colouring agents.

Further indications should be given, such as the special route of administration of a dosage form and, whenever relevant, the shelf-life.

Additional information
Information may be given concerning certain characteristics of a substance such as polymorphism, hygroscopicity or stability. Information may also be provided on precautions, special routes of administration, and the usual strengths for dosage forms[1].

General requirements
A substance or a dosage form stated to be of pharmacopoeial quality must comply with all the requirements set out in *The international pharmacopoeia*. A monograph is to be interpreted in accordance with any general notice or monograph or with any general method text that is contained in this edition and that is applicable to that monograph.

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[1] *The selection and use of essential medicines.* Report of the WHO Expert Committee. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 933). See also the current edition of the WHO Model Formulary and other publications available on the WHO Medicines website (http://www.who.int/medicines).

General methods of analysis that are commonly used in carrying out the tests and assays included in the monographs of the International Pharmacopoeia are described in the section on "Methods of Analysis". In some cases a specific cross-reference to the method required is provided within the monograph text. Examples include references to Spectrometry in the infrared region, 1.14.4 High performance liquid chromatography and 2.2.3 Limit test for heavy metals. In other cases, where the relevant method can be inferred from the title of the test, no explicit cross-reference is given. Examples include tests for Specific optical rotation, Sulfated ash, Loss on drying and pH value. Whether or not a specific cross-reference is included, the requirements of the monographs of the International Pharmacopoeia are to be interpreted in relation to the relevant method of analysis.

Limits are evaluated on the basis of normal analytical practice. Variations in manufacture and/or compounding, any possible deterioration, and normal analytical errors are taken into consideration. However, further tolerances beyond the limits stated are not permitted.

Limits are generally expressed in terms of the equivalent content in per cent (%) of the chemical formula for a given substance, as determined in the assay.

The requirements for biological substances and preparations such as certain antibiotics, if determined biologically, are given in International Units (IU) per milligram (mg). The material is not of pharmacopoeial quality if the upper fiducial limit of error is less than the stated potency, with fiducial limits of error based on a probability of 95% ($P = 0.95$).

Where it is specified that the results are to be calculated with reference to the dried or anhydrous substance, the determination of loss on drying or water is to be carried out using the test conditions indicated in the monograph.

Identity tests

Identity tests are provided to verify the identity of the substance described in the monograph, and analysts will need to decide on the extent of testing that they are able to carry out based on the instruments available to them.

It is generally recognized that the infrared spectrum provides the best method of identification because of the uniqueness of a well developed fingerprint region of the spectrum for a given active ingredient. Wherever possible, infrared spectrum characteristics are used as the principal test of identification. Further identification tests provided in an individual monograph, considered together, are intended to provide verification of identity, should the use of an infrared spectrophotometer be precluded.

It should further be noted that, whenever a melting temperature is provided under "Identity tests", only an approximate value is given, since no exact reproduction of the quoted temperature is necessary.

Examination in ultraviolet light

When examination in ultraviolet light is required, an ultraviolet lamp having a maximum output of approximately 254 nm or 365 nm is to be used.

Clarity of solution

The determination of the clarity of solution is carried out as described under 1.11 Colour of liquids using a black background. The source of light must be such that opalescence standard TS2 can be readily distinguished from water. A solution is considered clear if its opalescence is not more pronounced than that of opalescence standard TS2.

Colourless solution

A solution is considered colourless if it is not more intensely coloured than any of the standard colour solutions Bn0, Yw0, Gn0, or Rd0. The matching is made with the solution of most appropriate hue as described under 1.11 Colour of liquids.

Loss on drying

In order to determine the loss on drying, 1.0 g of the substance – unless otherwise specified – is dried under the conditions indicated.

The desiccants mentioned may be replaced by other substances of equivalent desiccant capacity.

The expression "dry to constant mass" means that the drying process should be continued until the results of two consecutive weighings do not differ by more than 0.5 mg, the second weighing being made after an additional hour of drying under the prescribed conditions. The expression "ignite to constant mass" has a similar meaning, the second weighing following further ignition. The expression "under reduced pressure" means that the drying process is carried out over phosphorus pentoxide, at a pressure not exceeding 0.6 kPa at room temperature, unless otherwise stated.

Tests and assays

Alternative methods are given to be used in cases where the required instruments are not available.

Tests and assays are normally carried out at room temperature (between 15 and 25 °C, or up to 30 °C in some climatic zones), unless otherwise indicated.

Any glassware used in pharmacopoeial tests and assays should be of suitable quality.[1]

When a water-bath is referred to in the text, boiling water of about 100 °C is to be used, unless a specific water temperature is given.

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[1] The following requirements concerning the quality of glass were established by the International Organization for Standardization:

— Glass – Hydrolytic resistance of glass grains at 98 °C – Method of test and classification, Ref. No. ISO 719-1985 (E).
— Glass – Hydrolytic resistance of glass grains at 121 °C – Method of test and classification, Ref. No. ISO 720-1985 (E).
— Glass-ware – Hydrolytic resistance of the interior surfaces of glass containers – Methods of test, Ref. No. ISO 4802-1988 (E).

Unless otherwise specified, all solutions indicated in the tests and assays are prepared with distilled or demineralized water complying with the monograph for Purified water.

Indicators for visual determination of pH values

Alternative indicators and indicator papers can be used in the tests and assays for the determination of the pH value or of its change by visual inspection provided that they have the same sensitivity in the chosen pH range.

Accuracy and Precision

(a) Expression of concentration

The symbol "%" is normally used to express the concentration of solids in liquids, the number of grams of solute in 100 ml of product (mass per volume). If indicated, the following expressions are used:

% *m/m* for the number of grams of solute in 100 g of product
% *v/v* for the number of millilitres of solute in 100 ml of product
% *v/m* for the number of millilitres of solute in 100 g of product.

The concentration expressed as "mol/l" refers to the number of moles of the stated solute in sufficient water, or other vehicle, to produce 1000 ml.

(b) Quantities

In assays and tests with numerical limits, the quantity stated to be taken for examination is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result is calculated from this exact quantity. Regents are measured and the procedures are carried out with an accuracy commensurate with the degree of precision implied by the requirement stated for the assay or the limit specified for the test. In tests where the limit is not numerical (for example, in comparative tests such as that for Clarity and colour of solution), the stated quantity is taken for examination. Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision corresponds to plus or minus 5 units after the last figure stated. For example,

a value of	20 is not less than	19.5 and not greater than	20.5,
	20.0 is not less than	19.95 and not greater than	20.05,
	2.0 is not less than	1.95 and not greater than	2.05,
	0.20 is not less than	0.195 and not greater than	0.205.

(c) pH value

pH values are indicated in a manner similar to that given for quantities and volumes.

(d) Temperature measurement

Temperature measurement is indicated in a manner similar to that given for quantities.

Storage conditions given in general terms refer to the following equivalent temperatures:

in a refrigerator	2 to 8 °C
cold or cool	8 to 15 °C
room temperature	15 to 25 °C, or up to 30 °C in some climatic zones.

Calculation of results

The results of tests and assays should be calculated to one decimal place more than indicated in the requirement and then rounded up or down as follows:

— if the last figure calculated is 5 to 9, the preceding figure is increased by 1;
— if the last figure is 4 or less, the preceding figure is left unchanged.

Other calculations – for example, in the standardization of volumetric solutions – are carried out in a similar manner.

Impurities

In certain tests, the concentration of impurity is given in parentheses either as a percentage or in parts per million by weight (ppm). In chromatographic tests such concentrations are stated as a percentage irrespective of the limit.

In chromatographic tests in which a comparison is made between spots or peaks in chromatograms obtained with solutions of different concentrations of the test substance, the percentage given in parentheses indicates an impurity limit expressed in terms of a nominal concentration of the medicinal substance itself.

In all cases where an impurity limit is given in parentheses, the figures given are approximations for information only; conformity with the requirements is determined on the basis of compliance or otherwise with the stated test.

A list of all known and potential impurities that have been shown to be controlled by the tests in a monograph may be given for information at the end of the monograph.

Patents and trademarks

The inclusion in *The International Pharmacopoeia* of any product subject to actual or potential patent or similar rights, or the inclusion of any name that is a trademark in any part of the world does not and shall not be deemed to imply or convey permission, authority, or licence to exercise any right or privilege protected by such patent or trademark, including licence to manufacture, without due permission, authority, or licence from the person or persons in whom such rights and privileges are vested.

Reference to a particular trade name in the description of certain materials used in assays and tests does not imply that other equivalent materials are not also suitable.

Reagents, reference substances, test solutions, and volumetric solutions

Names of reagents (R and IR), reference substances (RS), test solutions (TS), volumetric solutions (VS), and culture media (Cm) are described in the section on Reagents. Reagents used in tests and assays are not intended for therapeutic use.

Reference substances

(a) Chemical

International Chemical Reference Substances are established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs.

Directions for use and the analytical data required for the tests specified in *The International Pharmacopoeia* are given in the certificates enclosed with the substances when distributed. More detailed analytical reports on the substances may be obtained on request from the WHO Collaborating Centre for Chemical Reference Substances at the address given below.

International Chemical Reference Substances may also be used in tests and assays not described in *The International Pharmacopoeia*. However, responsibility for assessing the suitability of the substances then rests with the user or with the pharmacopoeia commission or other authority that has prescribed their use.

It is generally recommended that the substances be stored protected from light and preferably at a temperature of about +5 °C. When special storage conditions are required, this is stated on the label or in the accompanying leaflet.

The stability of the International Chemical Reference Substances kept at the Collaborating Centre is monitored by regular re-examination, and materials that have deteriorated are replaced by new batches when necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre. The list published in the 2004 annual report is provided in the section on Supplementary information. The list current at any particular time is available on the WHO Medicines website or may be obtained on request from the centre.

Orders for International Chemical Reference Substances should be sent to:

- WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Centrallaboratoriet, ACL, Prismávägen 2, SE-141 75 Kungens Kurva, Sweden; Fax: +46 8 740 60 40; E-mail: who.apl@apoteket.se

International Chemical Reference Substances are supplied only in the standard packages indicated in the list.

(b) Biological

The primary purpose underlying the establishment of International Biological Standards is to provide a means of ensuring uniformity throughout the world in the designation of the activity or specificity of preparations that are used in the prophylaxis, therapy, or diagnosis of human diseases, which cannot be expressed directly in terms of chemical and physical quantities[1].

Requests for international biological reference materials should be addressed directly to the following custodian laboratory, together with a brief summary on the intended use. The catalogue of International Biological Reference Preparations current at any particular time is available on the WHO Biologicals website.

- International Laboratory for Biological Standard, National Institute for Biological Standards and Control (NIBSC) South Mimms, Potters Bar, Herts EN6 3QH, England; Tel. +44 1707 646399; Telex 21911 NIBSAC G; Fax +44 1707 646977.

Reference spectra

International Infrared Reference Spectra are intended to be used to confirm the identity of specific substances. These spectra can be obtained from:

- WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Centrallaboratoriet, ACL, Prismavägen 2, SE-141 75 Kungens Kurva, Sweden; Fax +46 8 740 60 40. E-mail: who.apl@apoteket.se

[1] More information may be found in: *Recommendations for the preparation, characterization and establishment of international and other biological reference standards (Revised 2004)* Annex 2 to 55th Report of the WHO Expert Committee on Biological Standardization, Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 932).

Appendices to the General Notices

Abbreviations and symbols

$[\alpha]$	Angle of rotation in polarized light.
$[\alpha]_D^{20\,°C}$	Specific optical rotation, angle of rotation (α) of a liquid or of a substance in solution, measured in degrees (°) of rotation at the wavelength of the sodium D-line (589.3 nanometres) and at a temperature of 20 ± 0.5 °C. For liquids it is calculated with reference to a path length of 100 millimetres and divided by the relative density at 20 °C; for substances in solution it is calculated with reference to a path length of 100 millimetres of a solution containing 1 gram of the substance per millilitre.
A	Absorbance, extent of radiation absorbed by a solution expressed in logarithm, decimal, of the reciprocal of the transmittance.
$A_{1\,cm}^{1\%}$	Specific absorbance, absorbance of a solution containing 1 gram of substance in 100 millilitres of solution (1% solution) and measured at a path length of 1 centimetre.
ATCC	American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA.
CAS Reg.	Registration Number established by the Chemical Abstracts Service of the American Chemical Society.
C.I.	Colour Index Number (British).
CIP	Collection de Bactéries de l'Institut Pasteur, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, France.
Cm	Culture medium (see "Reagents, test solutions, and volumetric solutions").
d	Relative density, dimensionless, ratio of the density (r) of a substance to that of a reference substance under specified conditions.

d_{20}^{20}	Relative density, ratio of the mass of a given volume of the substance to that of an equal volume of water, both at 20 °C.
$E_{1\,cm}^{1\%}$	This symbol has been replaced by $A_{1\,cm}^{1\%}$.
IU	International Units.
mol/l	Concentration, amount of substance of solute per 1000 millilitres of solution.
n	Refractive index for electromagnetic radiation in a given medium, dimensionless, ratio of the sine of the angle of incidence of electromagnetic radiation on a medium to the sine of its angle of refraction in the medium.
n_D^{20}	Refractive index, value measured at the wavelength of the sodium D-line (589.3 nanometres) and at a temperature of 20 ± 0.5 °C.
NCIMB	National Collections of Industrial and Marine Bacteria Ltd, 23 St Machar Drive, Aberdeen AB24 3RY, Scotland.
NCTC	National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England.
NCYC	National Collection of Yeast Cultures, AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA, England.
P	Probability of an event in statistical estimations.
pH	Dimensionless, physical quantity expressing the acidity or alkalinity of a solution, measured as the negative logarithm of the hydrogen-ion concentration or hydrogen-ion activity expressed in moles per litre. The scale, 0–14, represents the acidity and alkalinity, 7 indicating the neutral state, numbers below 7 an increasing acidity, and numbers above 7 an increasing alkalinity.
ρ	Mass density, mass divided by volume.
ρ_{20}	Mass density, the mass of one unit volume of the substance, expressed in kilograms per cubic metre (in *The International Pharmacopoeia* as grams per millilitre) measured at a temperature of 20 °C.
R	Reagent (see "Reagents, test solutions, and volumetric solutions").

R_f	Ratio of fronts, related to fronts. In paper or thin-layer chromatography, ratio of the distance travelled by the substance to that travelled by the mobile phase.
R_r	In chromatography, ratio of the distances travelled by the substance to that of the reference substance.
RS	Reference substance (see "Reagents, test solutions, and volumetric solutions").
SI	International System of Units (*Système international d'Unités*).
TS	Test solution (see "Reagents, test solutions, and volumetric solutions").
VS	Volumetric solution (see "Reagents, test solutions, and volumetric solutions").

Units of measurement

The names and symbols for units of measurement used by the *International Pharmacopoeia* are those of the *Système international d'Unités* (International System of Units) (SI), a practical system of units that has been developed through the efforts of the General Conference of Weights and Measures (CGPM) and other international organizations. The 11th General Conference (1960) adopted the international abbreviation SI for this system of units[1].

The SI units used in the third edition of the *International Pharmacopoeia,* as well as their multiples and submultiples, were in many cases identical to the units used for the respective units of measurement in the second edition. In other cases, however, the SI had introduced differently defined units; this was especially true for derived units. In such situations, to promote better understanding of the procedures and limits related to quality requirements, the third edition of the *International Pharmacopoeia* gave, in addition to the SI units, the units previously used in the second edition, together with appropriate conversion of numerical values. Where included, this information has been retained in the fourth edition.

The following multiplicative prefixes, which indicate decimal multiples and submultiples of the SI units, are used in the *International Pharmacopoeia:*

giga	(G)	10^{9}
mega	(M)	10^{6}
kilo	(k)	10^{3}
centi	(c)	10^{-2}
milli	(m)	10^{-3}
micro	(μ)	10^{-6}
nano	(n)	10^{-9}
pico	(p)	10^{-12}

The use of these prefixes is illustrated by the following units, multiples and submultiples, that are employed in the third edition of the *International Pharmacopoeia*:

[1] The definitions of the units used in the International System are given in the brochure "Le Système international d'unités" published by the Bureau International des Poids et Mesures, (BIPM) Pavillion de Breteuil, F-92310 Sèvres. The most recent edition was published in 1998 and a Supplement in 2000. For more information, consult the BIPM website (http://www.bipm.fr).

Units of length

metre	(m)
centimetre	(cm)
millimetre	(mm)
micrometre	(μm)
nanometre	(nm)

Units of volume (capacity)

litre	(l) =	$1000\,cm^3$
millilitre	(ml) =	$1\,cm^3$
microlitre	(μl) =	$0.001\,cm^3$

Units of temperature

Kelvin	(K)
degree Celsius	(°C)

Units of mass

kilogram	(kg)
gram	(g)
milligram	(mg)
microgram	(μg)
nanogram	(ng)

Units of time

year	(a)
day	(d)
hour	(h)
minute	(min)
second	(s)
millisecond	(ms)
microsecond	(μs)

Units of pressure

kilopascal	(kPa)
pascal	(Pa)

The following non-SI units of pressure are also used in some special cases:

pound-force per square inch (lbf/in^2 or, incorrectly, psi)	≈ 0.69 kPa
millimetre of mercury (mmHg)	≈ 133 Pa

Units of radioactivity[1]

gigabecquerel (GBq)	= 27.03 mCi
megabecquerel (MBq)	= 27.03 μCi
becquerel (Bq)	= 27.03 pCi
curie (Ci)	= 37 GBq
millicurie (mCi)	= 37 MBq
microcurie (μCi)	= 37 kBq

Units of electric current

ampere	(A)
milliampere	(mA)
nanoampere	(nA)

Units of electric potential

volt	(V)
millivolt	(mV)

Unit of resistance

ohm	(Ω)

[1] The definition of units of radioactivity is given under "Radiopharmaceuticals".

Names, symbols, and relative atomic masses of certain elements

The relative atomic mass (the term atomic weight was previously used) given in the table below is scaled relatively to the isotope ^{12}C taken as 12 exactly and is given to 4 significant figures. Only the elements most commonly encountered in pharmaceutical analysis are included.

Most elements have more than one naturally occurring isotope and the variation in the relative abundance of these isotopes influences the precision with which the relative atomic mass of an element in nature can be quoted. Among the elements included in the table, the relative atomic mass of samples in nature of boron, calcium, lead, strontium and sulfur may differ by more than one in the fourth significant figure.

Name	*Symbol*	*Relative atomic mass*
Aluminium	Al	26.98
Antimony	Sb	121.75*
Arsenic	As	74.92
Barium	Ba	137.3
Bismuth	Bi	209.0
Boron	B	10.81
Bromine	Br	79.90
Cadmium	Cd	112.4
Calcium	Ca	40.08
Carbon	C	12.01
Cerium	Ce	140.1
Chlorine	Cl	35.45
Chromium	Cr	52.00
Cobalt	Co	58.93
Copper	Cu	63.55*
Fluorine	F	19.00
Gold	Au	197.0
Helium	He	4.003
Holmium	Ho	164.9
Hydrogen	H	1.008
Iodine	I	126.9
Iron	Fe	55.85*

Name	*Symbol*	*Relative atomic mass*
Lanthanum	La	138.9
Lead	Pb	207.2
Lithium	Li	6.941*
Magnesium	Mg	24.31
Manganese	Mn	54.94
Mercury	Hg	200.6*
Molybdenum	Mo	95.94
Nickel	Ni	58.71*
Nitrogen	N	14.01
Oxygen	O	16.00*
Phosphorus	P	30.97
Platinum	Pt	195.1*
Potassium	K	39.10*
Ruthenium	Ru	101.1*
Selenium	Se	78.96*
Silicon	Si	28.09*
Silver	Ag	107.9
Sodium	Na	22.99
Strontium	Sr	87.62
Sulfur	S	32.06
Thorium	Th	232.0
Tin	Sn	118.7*
Titanium	Ti	47.90*
Tungsten (Wolfram)	W	183.85*
Uranium	U	238.0
Vanadium	V	50.94*
Zinc	Zn	65.38
Zirconium	Zr	91.22

* Values are considered reliable to ±1 in the last digit or ±3 when followed by an asterisk.

Monographs

Pharmaceutical substances

Pharmaceutical substances

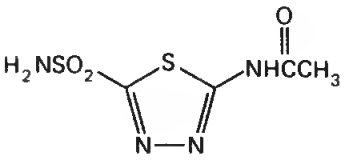
ACETAZOLAMIDUM

ACETAZOLAMIDE

Molecular formula. $C_4H_6N_4O_3S_2$

Relative molecular mass. 222.2

Graphic formula.



Chemical name. *N*-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide; *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide; 5-acetamido-1,3,4-thiadiazole-2-sulfonamide; CAS Reg. No. 59-66-5.

Description. A white, or almost white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Carbonic anhydrase inhibitor.

Storage. Acetazolamide should be kept in a well-closed container.

Requirements

Definition. Acetazolamide contains not less than 99.0% and not more than 101.0% of $C_4H_6N_4O_3S_2$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from acetazolamide RS or with the *reference spectrum* of acetazolamide.

B. Dissolve 25 mg in 5 ml of water, add 0.15 ml of sodium hydroxide (1 mol/l) VS and 0.1 ml of copper(II) sulfate (80 g/l) TS; a bluish green colour or precipitate is formed.

C. Triturate 0.5 g with a mixture of 5 ml of water and 1 ml of sodium hydroxide (1 mol/l) VS, add 0.2 g of zinc R powder and 0.5 ml of hydrochloric acid (~420 g/l) TS; hydrogen sulfide is evolved and may be detected by its odour (proceed with caution), or by the use of filter paper soaked in lead acetate (80 g/l) TS which turns black on exposure.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metal content according to Method A; not more than 20 µg/g.

Sulfates. Dissolve 1.0 g in 40 ml of water, warm to 70 °C for 5 minutes, cool and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.5 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. Shake 1 g with 50 ml of water for 5 minutes; pH of the suspension, 4.0–6.0.

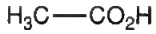
Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 30 volumes of dioxan R, 30 volumes of 2-propanol R, 20 volumes of ammonia (~35 g/l) TS, 10 volumes of toluene R, and 10 volumes of xylene R as the mobile phase. Apply separately to the plate 20 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 5.0 mg of the test substance per ml and (B) 0.050 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.45 g, accurately weighed, in 90 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 22.22 mg of $C_4H_6N_4O_3S_2$.

ACIDUM ACETICUM

ACETIC ACID

This monograph covers three concentrations, namely glacial acetic acid, acetic acid and dilute acetic acid.



$C_2H_4O_2$, Glacial acetic acid

Relative molecular mass. Glacial acetic acid, 60.05

Chemical name. Acetic acid; CAS Reg. No. 64-19-7.

Description. Clear, colourless liquids. At a temperature below 15 °C Glacial acetic acid may occur as a translucent, crystalline mass; odour, characteristic and pungent.

Miscibility. Miscible with water, ethanol (~750 g/l) TS, and glycerol R.

Category. Acidifying agent.

Storage. Acetic acid should be kept in a tightly closed container.

Additional information. Glacial acetic acid is flammable and should be handled with care. Congealing point, about 15 °C.

Relative densities. d_{20}^{20}

– Glacial acetic acid	1.050
– Acetic acid	1.041
– Dilute acetic acid	1.005

Requirements

Glacial acetic acid contains not less than **99.0% *m/m*** and not more than the equivalent of **100.5% *m/m*** of $C_2H_4O_2$.

Acetic acid contains not less than **32.5% *m/m*** and not more than the equivalent of **33.5% *m/m*** of $C_2H_4O_2$.

Dilute acetic acid contains not less than **5.7% *m/m*** and not more than the equivalent of **6.3% *m/m*** of $C_2H_4O_2$.

Identity tests

A. Strongly acid, when diluted.

B. Transfer to a test-tube either 0.1 ml of Glacial acetic acid with 1 ml of water, or take 1 ml of Acetic acid or of Dilute acetic acid; to each add 1 ml of ethanol (~750 g/l) TS and 1 ml of sulfuric acid (~1760 g/l) TS, and heat the mixture to boiling; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

C. Dilute either 0.3 ml of Glacial acetic acid with 3 ml of water or 1 ml of Acetic acid with 2 ml of water, or take 3 ml of Dilute acetic acid; neutralize each of them with ammonia (~100 g/l) TS. Then add 0.5 ml of lanthanum nitrate (30 g/l) TS, 0.1 ml of iodine TS, and 0.05 ml of ammonia (~100 g/l) TS. Heat the mixtures carefully to boiling and allow to stand; a dark blue colour appears.

Heavy metals. Take 3.5 ml of Glacial acetic acid, 10 ml of Acetic acid, or 20 ml of Dilute acetic acid. Evaporate each of the volumes to dryness on a water-bath. To each residue add 1.5 ml of hydrochloric acid (0.1 mol/l) VS, warm gently to dissolve, and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; Glacial acetic acid contains not more than 6 µg/g, Acetic acid not more than 2 µg/g, and Dilute acetic acid not more than 1 µg/g.

Chlorides. Take 3.5 ml and proceed with the test as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 70 µg/g.

Sulfates. Take 2 ml and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.24 mg/g.

Non-volatile residue. Evaporate a volume of known mass (not less than 10 g) to dryness on a water-bath and dry at 105 °C; the residue weighs not more than 0.1 mg/g.

Readily oxidizable substances. Dilute 5 ml of Glacial acetic acid with 10 ml of water and dilute again 5 ml of this solution with 20 ml of water, or use 5 ml of Acetic acid diluted with 20 ml of water, or use 25 ml of Dilute acetic acid. To each add 0.2 ml of potassium permanganate (0.02 mol/l) VS, and allow to stand for 30 seconds; the colour is not entirely discharged.

Assay. To about 2 g, accurately weighed, of Glacial acetic acid and 50 ml of water, or about 5 g, accurately weighed, of Acetic acid and 50 ml of water, or about 20 g, accurately weighed, of Dilute acetic acid and 30 ml of water, add 3 drops of phenolphthalein/ethanol TS and titrate with carbonate-free sodium hydroxide (1 mol/l) VS. Repeat the procedure without the acids being examined and make any necessary corrections.

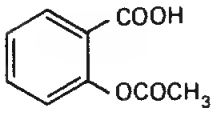
Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 60.05 mg of $C_2H_4O_2$.

ACIDUM ACETYLSALICYLICUM
ACETYLSALICYLIC ACID

Molecular formula. $C_9H_8O_4$

Relative molecular mass. 180.2

Graphic formula.



Chemical name. 2-(Acetyloxy)benzoic acid; 2-acetoxybenzoic acid; CAS Reg. No. 50-78-2.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Soluble in about 300 parts of water; freely soluble in ethanol (~750 g/l) TS; soluble in ether R.

Category. Analgesic; antipyretic.

Storage. Acetylsalicylic acid should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Acetylsalicylic acid is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Acetylsalicylic acid contains not less than 99.0% and not more than 100.5% of $C_9H_8O_4$, calculated with reference to the dried substance.

Identity tests

A. Heat 0.05 g in 2 ml of water for several minutes, cool, and add 1–2 drops of ferric chloride (25 g/l) TS; a violet-red colour is produced, which does not change on the addition of ethanol (~750 g/l) TS.

B. Boil 0.2 g with 4 ml of sodium hydroxide (~80 g/l) TS for about 3 minutes, cool, and add 5 ml of sulfuric acid (~100 g/l) TS; a white, crystalline precip-

itate is formed. Filter (keep the filtrate for test C), wash the precipitate with water and dry at 105 °C. Melting temperature, about 159 °C (salicylic acid).

C. Heat the filtrate from test B with 2 ml of ethanol (~750 g/l) TS and 2 ml of sulfuric acid (~1760 g/l) TS; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

Heavy metals. Use 1.0 g and 25 ml of acetone R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, procedure 2; determine the heavy metal content according to Method A; not more than 20 µg/g.

Solution in ethanol. A solution of 1.0 g in 10 ml of ethanol (~750 g/l) TS is clear and colourless.

Solution in alkali. A solution of 0.5 g in 10 ml of warm sodium carbonate (50 g/l) TS is clear.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R; it loses not more than 5.0 mg/g.

Salicylic acid. Dissolve 0.50 g in sufficient ethanol (~750 g/l) TS to produce 25 ml and transfer 10 ml to a comparison tube. Dissolve separately 0.040 g of salicylic acid R in sufficient water to produce 100 ml. Transfer 1 ml of this solution to a second comparison tube and add to it 10 ml of ethanol (~750 g/l) TS. Add water to both tubes to make 50 ml, followed by 1 ml of ferric ammonium sulfate TS1, mix and allow to stand for 1 minute. The violet colour of the test solution is not more intense than that of the reference solution when compared as described under 1.11 Colour of liquids; the salicylic acid content is not more than 2.0 mg/g.

Assay. To about 0.20 g, accurately weighed, add 50 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS, and boil under reflux for 10 minutes. Titrate the excess of alkali with sulfuric acid (0.05 mol/l) TS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 9.008 mg of $C_9H_8O_4$.

ACIDUM ALGINICUM
ALGINIC ACID

Chemical name. Alginic acid; CAS Reg. No. 9005-32-7.

Description. A white to yellowish white, fibrous powder; almost odourless.

Solubility. Slightly soluble in water; practically insoluble in most organic solvents; dissolves in solutions of alkali hydroxides.

Category. Tablet binder and disintegrant; viscosity-increasing agent; release-rate modifier.

Storage. Alginic acid should be kept in a well-closed container.

Additional information. Alginic acid has an equivalent mass of about 240. Attention should be paid to the microbiological quality since Alginic acid is of natural origin.

Requirements

Definition. Alginic acid is a polyuronic acid composed of D-mannuronic and L-guluronic acids and is obtained chiefly from algae belonging to the Phaeophyceae, mainly species of *Laminaria*.

Identity tests

A. Dissolve 30 mg in 5 ml of sodium hydroxide (0.1 mol/l) VS and add 1 ml of calcium chloride (55 g/l) TS; a voluminous, gelatinous precipitate is produced.

B. Dissolve 30 mg in 5 ml of sodium hydroxide (0.1 mol/l) VS and add 1 ml of sulfuric acid (~570 g/l) TS; a heavy, gelatinous precipitate is formed.

C. To 5 mg in a test-tube add 5 ml of water and 1 ml of a freshly prepared solution of naphthalene-1,3-diol R containing 1 g in 100 ml of ethanol (~750 g/l) TS. Heat the mixture to boiling, boil gently for 3 minutes, and cool to about 15 °C. Transfer the contents of the test-tube to a 30-ml separator with the aid of 5 ml of water and extract with 15 ml of diisopropyl ether R. For the blank repeat the procedure without the Alginic acid being examined. The red-violet colour produced in the extract is not darker than that obtained in the blank.

Heavy metals. For the preparation of the test solution ignite carefully in a crucible 1.0 g with 2 ml of nitric acid (~1000 g/l) TS and 5 drops of sulfuric acid (~1760 g/l) TS until white vapours evolve, then ignite completely. Cool, add

2 ml of hydrochloric acid (~420 g/l) TS, and evaporate slowly on a water-bath until dry. To the residue add 1 drop of hydrochloric acid (~420 g/l) TS and 10 ml of water, then add sufficient ammonia (~260 g/l) TS to render the solution slightly alkaline, adjust the pH to 3–4 with acetic acid (~60 g/l) TS, and dilute to 40 ml with water. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 40 µg/g.

Ash. Carry out the procedure as described under 4.1 Determination of ash and acid-insoluble ash; not more than 40 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 0.18 g/g.

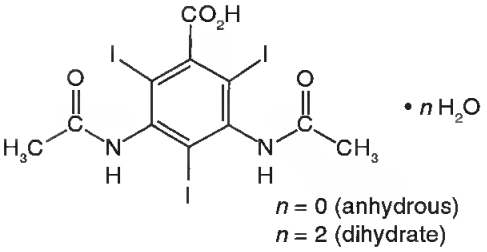
pH value. Disperse 3 g in 100 ml of water, pH 1.5–3.5.

Acid value. Suspend about 1 g, accurately weighed, in a mixture of 50 ml of water and 30 ml of calcium acetate (0.25 mol/l) VS. Shake thoroughly, allow to stand for 1 hour, and titrate with sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure without the Alginic acid being examined and make any necessary corrections. Proceed with the calculation as given in 4.6 Determination of acid value, using the number of ml of sodium hydroxide (0.1 mol/l) VS required as *a* and with reference to the dried substance; not less than 230.

ACIDUM AMIDOTRIZOICUM

AMIDOTRIZOIC ACID

Amidotrizoic acid, anhydrous
Amidotrizoic acid, dihydrate



$C_{11}H_9I_3N_2O_4$ (anhydrous)

$C_{11}H_9I_3N_2O_4$, $2H_2O$ (dihydrate)

Relative molecular mass. 613.9 (anhydrous); 649.9 (dihydrate).

Chemical name. 3,5-Diacetamido-2,4,6-triiodobenzoic acid; 3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid; CAS Reg. No. 117-96-4 (anhydrous).

3,5-Diacetamido-2,4,6-triiodobenzoic acid dihydrate; 3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid dihydrate; CAS Reg. No. 50978-11-5 (dihydrate).

Other name. Diatrizoic acid.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Very slightly soluble in water and ethanol (~750 g/l) TS; soluble in dimethylformamide R; sparingly soluble in methanol R; practically insoluble in ether R; dissolves in solutions of alkali hydroxides.

Category. Used in the preparation of meglumine amidotrizoate as a radiocontrast medium.

Storage. Amidotrizoic acid should be kept in a well-closed container, protected from light.

Labelling. The designation on the container of amidotrizoic acid should state whether the substance is the dihydrate or the anhydrous form.

Requirements

Amidotrizoic acid contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{11}H_9I_3N_2O_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. Previously dry the dihydrate of amidotrizoic acid at 105 °C for 4 hours. The infrared absorption spectrum is concordant with the spectrum obtained from amidotrizoic acid RS or with the *reference spectrum* of amidotrizoic acid.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 20 volumes of chloroform R, 10 volumes of methanol R, and 2 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in a mixture of 0.8 g of sodium hydroxide R dissolved in 1000 ml of methanol R containing (A) 1 mg of Amidotrizoic acid per ml and (B) 1 mg of amidotrizoic acid RS per ml. After removing the plate from the

chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Heat 0.5 g in a suitable crucible; violet vapours are evolved.

D. Use 10 mg; it yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a red-violet precipitate.

Heavy metals. Suspend 10 g in 10 ml of water and with stirring add slowly 1.5 ml of sodium hydroxide (~400 g/l) TS. When dissolved, adjust the pH to between 7.0 and 7.5 with sodium hydroxide (~80 g/l) TS or hydrochloric acid (~70 g/l) TS, and dilute with water to 20 ml. Use 2 ml of this solution and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 20 µg/g. (Keep the remaining solution for "Iodine and iodides".)

Halides. Dissolve 2.5 g in a mixture of 20 ml of water and 2.5 ml of ammonia (~100 g/l) TS. Add 20 ml of nitric acid (~130 g/l) TS, dilute with sufficient water to produce 100 ml, allow to stand for 15 minutes with occasional shaking, and filter. Eliminate the first 10 ml of the filtrate and proceed with 25 ml of the filtrate as described under "2.2.1 Limit test for chlorides"; the content of halides, expressed as chlorides, does not exceed 35 µg/g.

Iodine and iodides. Place 4 ml of the solution prepared above for "Heavy metals" in a 50-ml centrifuge tube and add 20 ml of water, 5 ml of toluene R, and 5 ml of sulfuric acid (~100 g/l) TS. Shake and centrifuge; the toluene layer shows no red colour. Add 2 ml of sodium nitrite (10 g/l) TS, shake well, and centrifuge. Similarly prepare a reference solution containing 0.5 mg of potassium iodide R in 22 ml of water. Any red colour in the toluene layer is no darker than that obtained with the reference solution (200 µg I/g).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 4 hours; anhydrous Amidotrizoic acid loses not more than 10 mg/g and the dihydrate loses not less than 45 mg/g and not more than 70 mg/g.

Primary aromatic amines. Dissolve about 0.2 g, accurately weighed, in a mixture of 5 ml of water and 1 ml of sodium hydroxide (~80 g/l) TS. Add 4 ml of sodium nitrite (10 g/l) TS and 10 ml of hydrochloric acid (1 mol/l) VS, shake, and allow to stand for 2 minutes. Add 5 ml of ammonium sulfamate (25 g/l) TS,

shake well, allow to stand for 1 minute, add 0.4 ml of 1-naphthol/ethanol TS and 15 ml of sodium hydroxide (~80 g/l) TS, and dilute with water to 50 ml.

Measure the absorbance at about 485 nm against a solvent cell containing the reagents prepared in a similar manner; the absorbance is not greater than 0.15.

Assay. Place about 0.3 g, accurately weighed, in a 125-ml conical flask and add 30 ml of sodium hydroxide (50 g/l) TS and 0.5 g of zinc R powder. Connect the flask to a reflux condenser and boil for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 ml of water into the flask, and filter the mixture. Rinse the flask and the filter thoroughly and add the rinse liquids to the filtrate. Add 5 ml of glacial acetic acid R and 1 ml of tetrabromophenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l) VS until the yellow precipitate just changes to green.

Each ml of silver nitrate (0.05 mol/l) VS is equivalent to 10.23 mg of $C_{11}H_9I_3N_2O_4$.

Additional requirement for Amidotrizoic acid for parenteral use

Complies with the monograph for "Parenteral preparations".

Pyrogens. Carry out the test as described under 3.5 Test for pyrogens, injecting, per kg of the rabbit's mass, a solution in sterile water R containing 0.6 g of Amidotrizoic acid in not more than 5 ml.

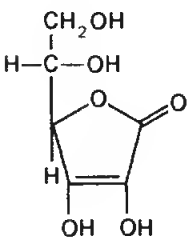
ACIDUM ASCORBICUM

ASCORBIC ACID

Molecular formula. $C_6H_8O_6$

Relative molecular mass. 176.1

Graphic formula.



Chemical name. L-Ascorbic acid; CAS Reg. No. 50-81-7.

Description. Colourless crystals or a white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiscorbutic.

Storage. Ascorbic acid should be kept in a tightly closed, non-metallic container, protected from light.

Additional information. Ascorbic acid in solution deteriorates rapidly in contact with air; it has an acid taste. Even in the absence of light, Ascorbic acid is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ascorbic acid contains not less than 99.0% and not more than 100.5% of $C_6H_8O_6$.

Identity tests

A. Dissolve 0.1 g in 2 ml of water, add a few drops of nitric acid (~130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a dark grey precipitate is produced.

B. Dissolve 0.04 g in 4 ml of water, add 0.1 g of sodium hydrogen carbonate R and about 20 mg of ferrous sulfate R, shake and allow to stand; a deep violet colour is produced, which disappears on the addition of 5 ml of sulfuric acid (~100 g/l) TS.

C. Melting temperature, about 190 °C with decomposition.

Specific optical rotation. Use a 50 mg/ml solution; $[\alpha]_D^{20\,°C}$ = +20.5 to +21.5 °.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Rd1 when compared as described under 1.11 Colour of liquids.

Readily carbonizable substances. Dissolve 0.10 g in 10 ml of sulfuric acid (~1760 g/l) TS. After 15 minutes the solution is not more intensely coloured than standard colour solutions Yw1 or Gn1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

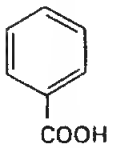
Assay. Dissolve about 0.20 g, accurately weighed, in a mixture of 25 ml of carbon-dioxide-free water R and 25 ml of sulfuric acid (~100 g/l) TS. Titrate the solution at once with iodine (0.05 mol/l) VS using starch TS as indicator, added towards the end of the titration, until a persistent blue colour is obtained. Each ml of iodine (0.05 mol/l) VS is equivalent to 8.806 mg of $C_6H_8O_6$.

ACIDUM BENZOICUM
BENZOIC ACID

Molecular formula. $C_7H_6O_2$

Relative molecular mass. 122.1

Graphic formula.



Chemical name. Benzenecarboxylic acid; CAS Reg. No. 65-85-0.

Description. Colourless, light, feathery crystals or a white, microcrystalline powder; odour, characteristic, faint.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS, ether R.

Category. Dermatological agent.

Storage. Benzoic acid should be kept in a well-closed container.

Requirements

Definition. Benzoic acid contains not less than 99.0% and not more than 100.5% of $C_7H_6O_2$, calculated with reference to the anhydrous substance.

Identity test
Boil 0.1 g with 0.1 g of calcium carbonate R1 and 5 ml of water, and filter; add a few drops of ferric chloride (25 g/l) TS to the filtrate; a beige coloured precipitate is produced.

Melting range. 121–124 °C.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in 25 ml of acetone R, add 2 ml of water and dilute to 40 ml with acetone R and mix; determine the heavy metal content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Chlorinated compounds and chlorides. Dissolve 0.35 g in 5 ml of sodium carbonate (50 g/l) TS, evaporate to dryness, and heat the residue until completely charred, keeping the temperature below 400 °C. Extract the residue with a mixture of 10 ml of water and 12 ml of nitric acid (~130 g/l) TS, and filter. Proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.7 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance and 25 ml of a solution composed of 1 volume of methanol R and 2 volumes of pyridine R as the solvent; the water content is not more than 7.0 mg/g.

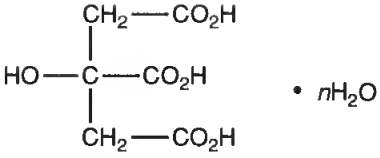
Readily oxidizable substances. Add 1.5 ml of sulfuric acid (~1760 g/l) TS to 100 ml of water, heat to boiling, and add, drop by drop, potassium permanganate (0.02 mol/l) VS until the pink colour persists for 30 seconds. Dissolve 1.0 g of the substance being examined in the hot solution and titrate with potassium permanganate (0.02 mol/l) VS to a pink colour that persists for 15 seconds; not more than 0.5 ml of potassium permanganate (0.02 mol/l) VS is required.

Assay. Dissolve about 0.25 g, accurately weighed, in 15 ml of ethanol (~750 g/l) TS, previously neutralized to phenol red/ethanol TS, add 20 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, using phenol red/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 12.21 mg of $C_7H_6O_2$.

ACIDUM CITRICUM

CITRIC ACID

Citric acid, anhydrous
Citric acid monohydrate.



$n = 0$ (anhydrous)
$n = 1$ (monohydrate)

$C_6H_8O_7$ (anhydrous)
$C_6H_8O_7,H_2O$ (monohydrate)

Relative molecular mass. 192.1 (anhydrous); 210.1 (monohydrate).

Chemical name. Citric acid; 2-hydroxy-1,2,3-propanetricarboxylic acid; CAS Reg. No. 77-92-9.

Citric acid monohydrate; 2-hydroxy-1,2,3-propanetricarboxylic acid monohydrate; CAS Reg. No. 5949-29-1.

Description. Colourless crystals or a white, crystalline powder; odourless or practically odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; sparingly soluble in ether R.

Category. Acidifying agent; buffer component.

Storage. Citric acid should be kept in a well-closed container.

Labelling. The designation on the container of Citric acid should state whether it is the monohydrate or the anhydrous form.

Additional information. Citric acid monohydrate effloresces in dry air.

Requirements

Citric acid contains not less than **99.5%** and not more than the equivalent of **101.0%** of $C_6H_8O_7$, calculated with reference to the anhydrous substance.

Identity test
A 20 mg/ml solution yields the reactions described under 2.1 General identification tests as characteristic of citrates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Barium. Dissolve 1 g in 7.8 ml of sodium hydroxide (~80 g/l) TS and dilute to 10 ml with water. Acidify half of this solution with sulfuric acid (~100 g/l) TS and allow to stand for at least 1 hour. Compare with the untreated portion of solution; it remains clear.

Oxalates. Dissolve 1 g in 10 ml of water, neutralize with ammonia (~100 g/l) TS, add 0.35 ml of hydrochloric acid (2 mol/l) VS, cool, and add 2 ml of calcium chloride (55 g/l) TS; no turbidity is produced.

Sulfates. Dissolve 0.1 g in 10 ml of water, add 1 ml of barium chloride (50 g/l) TS to which 1 drop of hydrochloric acid (~420 g/l) TS has been added; no turbidity is produced.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A.

— For the anhydrous form use 1 g; the water content is not more than 10 mg/g.

— For the monohydrate use 0.15 g; the water content is not less than 75 mg/g and not more than 90 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Assay. Dissolve about 1.5 g, accurately weighed, in 50 ml of carbon-dioxide-free water R and titrate with carbonate-free sodium hydroxide (1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure without the Citric acid being examined and make any necessary corrections.

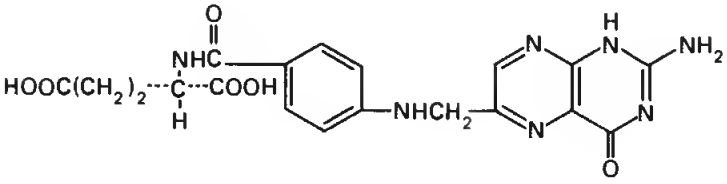
Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 64.03 mg of $C_6H_8O_7$.

ACIDUM FOLICUM
FOLIC ACID

Molecular formula. $C_{19}H_{19}N_7O_6$

Relative molecular mass. 441.4

Graphic formula.



Chemical name. *N*-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino]-benzoyl]-L-glutamic acid; *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamic acid; CAS Reg. No. 59-30-3.

Description. A yellow or yellowish orange, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; practically insoluble in ethanol (~750 g/l) TS, acetone R and ether R.

Category. Haemopoietic.

Storage. Folic acid should be kept in a well-closed container, protected from light.

Requirements

Definition. Folic acid contains not less than 96.0% and not more than 102.0% of $C_{19}H_{19}N_7O_6$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 15 µg/ml solution in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 380 nm, exhibits 3 maxima at about 256 nm, 283 nm, and 365 nm. The absorbances at these wavelengths are about 0.82, 0.80 and 0.28, respectively (preferably use 2-cm cells for the measurements and calculate the absorbances of 1-cm layers). The ratio of the absorbance of a 1-cm layer at 256 nm to that at 365 nm is between 2.80 and 3.00.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 2 volumes of 1-propanol R, 1 volume of ethanol (~750 g/l) TS, and 2 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS containing (A) 0.50 mg of the test substance per ml and (B) 0.50 mg of folic acid RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.15 g of the substance; the water content is not less than 70 mg/g and not more than 90 mg/g.

Free amines. The ratio of the absorbance A_T of the test solution T_2 to the absorbance A_B of the blank solution B_1, as measured in the assay, should be larger than 6.

Assay. Prepare the test solution T by dissolving about 0.050 g, accurately weighed, in 50 ml of sodium hydroxide (~80 g/l) TS, mixing and diluting with sodium hydroxide (~80 g/l) TS to 100 ml.

Transfer 30.0 ml of the test solution to a 100-ml volumetric flask (test solution T_1), and a second aliquot of 30.0 ml of the test solution to a second 100-ml volumetric flask (blank B_1). To both solutions, T_1 and blank B_1 add 20 ml of hydrochloric acid (~70 g/l) TS and dilute them both with water to volume. Retain blank solution B_1. To 60 ml of the test solution T_1 add 0.5 g of zinc R powder, and allow to stand, shaking frequently, for 20 minutes. Filter the mixture through a dry filter paper, discard the first 10 ml of the filtrate, and dilute 10 ml of the subsequent filtrate with water to 100 ml (test solution T_2).

Into three separate 25-ml volumetric flasks place 5.0 ml each of test solution T_2, of blank solution B_1 and of water (solution B_2), add to each of them 1 ml of water, 1 ml of hydrochloric acid (~70 g/l) TS, and 1 ml of sodium nitrite (1 g/l) TS, mix well and allow to stand for 2 minutes. Then add to each of them 1 ml of ammonium sulfamate (5 g/l) TS, mix thoroughly, allow to stand for 2 minutes, add 1 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS, shake, allow to stand for 10 minutes, and dilute with water to volume.

Measure the absorbance of the test solution T_2 and of the blank solution B_1 against a solvent cell containing solution B_2 at the maximum of about 550 nm; designate these as A_T and A_B, respectively.

Carry out a similar procedure using folic acid RS and designate the respective absorbances as A_S and A_{BS}.

Calculate the content of $C_{19}H_{19}N_7O_6$ in terms of the percentage of anhydrous substance in the test substance, using the formula: $100(10\ A_T - A_B)/(10\ A_S - A_{BS})$, if necessary multiplying the result by the declared content (%) of $C_{19}H_{19}N_7O_6$ in the chemical reference substance.

ACIDUM HYDROCHLORICUM
HYDROCHLORIC ACID

HCl

Relative molecular mass. 36.46

Chemical name. Hydrochloric acid; CAS Reg. No. 7647-01-0.

Description. A clear, colourless, fuming liquid; odour, pungent.

Miscibility. Miscible with water.

Category. Acidifying agent.

Storage. Hydrochloric acid should be kept in a tightly closed container, preferably in a cool place.

Additional information. The fumes and odour disappear when the acid is diluted with 2 volumes of water.

Mass density: ρ_{20} = about 1.18 g/ml.

Requirements

Hydrochloric acid contains not less than **35.0%** ***m/m*** and not more than the equivalent of **38.0%** ***m/m*** of HCl.

Identity tests

A. It is strongly acid.

B. Use 0.1 ml; it yields the reactions described under 2.1 General identification tests as characteristic of chlorides.

C. Allow a glass stick wetted with ammonia (~100 g/l) TS to come near the surface of Hydrochloric acid; white fumes are evolved.

Heavy metals. For the preparation of the test solution evaporate 4 g to dryness on a water-bath, add 2 ml of acetic acid (~60 g/l) PbTS, dilute to 40 ml and mix; determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 5 µg/g.

Arsenic. Dilute 4.3 ml to 10 ml with water. Use 1 ml and proceed as described under 2.2.5 Limit test for arsenic; not more than 2 µg/g.

For the following three tests mix 1 volume of Hydrochloric acid with 2 volumes of water:

Bromides and iodides. To 10 ml add 1 ml of chloroform R and add cautiously, a drop at a time with constant stirring, chlorine TS which has been diluted with an equal volume of water; the chloroform remains free from even a transient yellow, orange, or violet colour.

Free bromine and chlorine. To 10 ml add 1 ml of potassium iodide (80 g/l) TS and 1 ml of chloroform R, and shake the mixture; the chloroform remains free from any violet colour for at least 1 minute.

Sulfites. Mix 3 ml with 5 ml of water and add 5 drops of barium chloride (50 g/l) TS and 2 drops of iodine (0.05 mol/l) VS; no turbidity is produced and the colour of the iodine is not completely discharged.

Sulfates. To 20 ml add 40 mg of sodium hydrogen carbonate R and evaporate to dryness on a water-bath; dissolve the residue in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 20 µg/g.

Residue on ignition. Place 10 g in a porcelain dish and evaporate to dryness on a water-bath. Ignite the residue to constant mass; not more than 0.1 mg/g.

Assay. Add about 1.5 ml, accurately weighed, to a tared glass-stoppered flask containing 20 ml of water; then add 25 ml of water and titrate with sodium hydroxide (1 mol/l) VS, using methyl red/ethanol TS as indicator.

Each ml of sodium hydroxide (1 mol/l) VS is equivalent to 36.46 mg of HCl.

ACIDUM HYDROCHLORICUM DILUTUM
DILUTE HYDROCHLORIC ACID

Description. A colourless liquid; odourless.

Category. Acidifying agent.

Storage. Dilute hydrochloric acid should be kept in a rightly closed container.

Requirements

Dilute hydrochloric acid contains not less than **9.5% *m/m*** and not more than the equivalent of **10.5% *m/m*** of HCl.

Identity tests

A. It is strongly acid.

B. Use 0.5 ml; it yields the reactions described under 2.1 General identification tests as characteristic of chlorides.

Mass density. ρ_{20} = 1.043–1.049 g/ml.

Heavy metals. For the preparation of the test solution evaporate 4 g to dryness on a water-bath, add 2 ml of acetic acid (~60 g/l) PbTS, dilute to 40 ml and mix; determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 5 µg/g.

Arsenic. Dilute 17 ml to 20 ml with water. Use 2 ml and proceed as described under 2.2.5 Limit test for arsenic; not more than 0.5 µg/g.

Bromides and iodides. To 10 ml add 1 ml of chloroform R and add cautiously, a drop at a time with constant stirring, chlorine TS which has been diluted with an equal volume of water; the chloroform remains free from even a transient yellow, orange, or violet colour.

Free bromine and chlorine. To 10 ml add 1 ml of potassium iodide (80 g/l) TS and 1 ml of chloroform R, and shake the mixture; the chloroform remains free from any violet colour for at least 1 minute.

Sulfites. Mix 3 ml with 5 ml of water and add 5 drops of barium chloride (50 g/l) TS and 2 drops of iodine (0.05 mol/l) VS; no turbidity is produced and the colour of the iodine is not completely discharged.

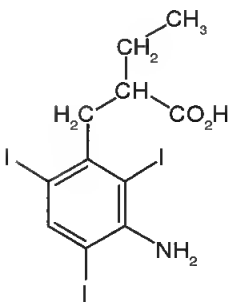
Sulfates. To 90 ml add 40 mg of sodium hydrogen carbonate R and evaporate to dryness on a water-bath; dissolve the residue in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 5 µg/g.

Residue on ignition. Place 10 g in a porcelain dish and evaporate to dryness on a water-bath. Ignite the residue to constant mass; not more than 0.1 mg/g.

Assay. Add about 2 ml, accurately weighed, to 20 ml of water and titrate with sodium hydroxide (1 mol/l) VS, using methyl red/ethanol TS as indicator.

Each ml of sodium hydroxide (1 mol/l) VS is equivalent to 36.46 mg of HCl.

ACIDUM IOPANOICUM
IOPANOIC ACID



$C_{11}H_{12}I_3NO_2$

Relative molecular mass. 570.9

Chemical name. 3-Amino-α-ethyl-2,4,6-triiodohydrocinnamic acid; 3-amino-α-ethyl-2,4,6-triiodobenzenepropanoic acid; CAS Reg. No. 96-83-3.

Description. A light yellowish white powder; odour, faint, characteristic.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS, and acetone R; dissolves in solutions of alkali hydroxides.

Category. Radiocontrast medium.

Storage. Iopanoic acid should be kept in a tightly closed container, protected from light. Iopanoic acid is gradually affected by light.

Requirements

Iopanoic acid contains not less than **97.0%** and not more than the equivalent of **101.0%** of $C_{11}H_{12}I_3NO_2$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from iopanoic acid RS or with the *reference spectrum* of iopanoic acid.

B. Heat strongly 0.05 g in a suitable crucible; violet vapours are evolved.

C. Melting temperature, about 155 °C with decomposition.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Iodides. Dissolve 0.8 g in a minimum quantity of sodium hydroxide (10 g/l) TS, dilute to 10 ml with water, add drop by drop nitric acid (~130 g/l) TS until complete precipitation is obtained, then add an excess of 3 ml. Filter, and wash the precipitate with 5 ml of water; to the filtrate add 1 ml of hydrogen peroxide (~330 g/l) TS and 1 ml of chloroform R, and shake. To serve as a reference solution, treat similarly 2 ml of iodide standard (20 µg I/ml) TS with 3 ml of nitric acid (~130 g/l) TS and sufficient water to equal the volume of the solution to be tested. Any red-violet colour in the chloroform layer is no darker than that obtained from the reference solution.

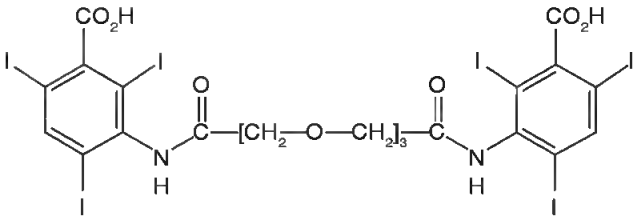
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 1 hour; it loses not more than 10 mg/g.

Assay. Place about 0.4 g, accurately weighed, in a 125-ml conical flask and add 30 ml of sodium hydroxide (50 g/l) TS and 0.5 g of zinc R powder. Connect the flask to a reflux condenser and boil for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 ml of water into the flask, and filter the mixture. Rinse the flask and the filter thoroughly and add the rinsing to the filtrate. Add 5 ml of glacial acetic acid R and 1 ml of tetrabromophenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l) VS until the yellow precipitate just changes to green.

Each ml of silver nitrate (0.05 mol/l) VS is equivalent to 9.516 mg of $C_{11}H_{12}I_3NO_2$.

ACIDUM IOTROXICUM
IOTROXIC ACID



$C_{22}H_{18}I_6N_2O_9$

Relative molecular mass. 1215.8

Chemical name. 3,3′-[Oxybis(ethyleneoxymethylenecarbonylimino)]bis[2,4,6-triiodobenzoic acid]; 3,3′-[oxybis[2,1-ethanediyloxy(1-oxo-2,1-ethanediyl) imino]]-bis[2,4,6,-triiodobenzoic acid]; CAS Reg. No. 51022-74-3.

Description. An almost white powder.

Solubility. Practically insoluble in water, benzene R, and ether R; freely soluble in methanol R and dimethylformamide R; dissolves in solutions of alkali hydroxides.

Category. Used in the preparation of meglumine iotroxate as a radiocontrast medium.

Storage. Iotroxic acid should be kept in a well-closed container, protected from light.

Requirements

Iotroxic acid contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{22}H_{18}I_6N_2O_9$, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from iotroxic acid RS or with the *reference spectrum* of iotroxic acid.

B. Heat 0.05g with 2ml of sulfuric acid (~1760g/l) TS in a suitable crucible; violet vapours are evolved.

Heavy metals. For the preparation of the test solution use 1.0 g, add 3 ml of meglumine (100 g/l) TS, and proceed as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Halides. Dissolve 10 g in 30 ml of meglumine (100 g/l) TS and titrate potentiometrically with silver nitrate (0.001 mol/l) VS. Each ml of silver nitrate (0.001 mol/l) VS is equivalent to 0.1269 mg of I; the content of halides, expressed as iodides, does not exceed 40 µg/g.

Solution in alkali. Dissolve 5 g in 5 ml of sodium hydroxide (~80 g/l) TS and add 2 ml of water; the solution is not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.4 g; the water content is not less than 10 mg/g and not more than 30 mg/g.

Foreign substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 62 volumes of chloroform R, 32 volumes of methanol R, 2 volumes of anhydrous formic acid R, and 6 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in methanol R containing (A) 0.1 g of Iotroxic acid per ml and (B) 0.5 mg of Iotroxic acid per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air at room temperature, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Primary aromatic amines. Transfer about 1 g, accurately weighed, to a 50-ml volumetric flask, dissolve in 2.5 ml of sodium hydroxide (1 mol/l) VS, and add 12.5 ml of water (= *solution A*). Dissolve 5 mg of 3-amino-2,4,6-triiodobenzoic acid RS in 0.2 ml of sodium hydroxide (0.1 mol/l) VS and dilute with sufficient water to produce 10 ml. Introduce 2 ml of this solution to a 50-ml volumetric flask and add 3 ml of water and 10 ml of sodium hydroxide (0.1 mol/l) VS (= *solution B*). For the blank solution, transfer 5 ml of water to a 50-ml volumetric flask and add 10 ml of sodium hydroxide (0.1 mol/l) VS.

Note: Strictly observe the instructions and proceed without delay using the three solutions concurrently.

Add 25 ml of dimethyl sulfoxide R to each solution, close the flasks, and swirl to mix. Place them in the dark in an ice-bath and allow to stand for 5 minutes.

Continue the procedure in the dark. Add while shaking 2 ml of hydrochloric acid (~420 g/l) TS and allow to stand again in the ice-bath for 5 minutes. Add while shaking 2 ml of freshly prepared sodium nitrite (20 g/l) TS. Using a stop-watch readable to 1 second, start the timing and allow to stand in the ice-bath for exactly 5 minutes. Add 1 ml of freshly prepared sulfamic acid (80 g/l) TS, start the timing again, shake until no more gas evolves, and allow to stand in the ice-bath for exactly 5 minutes. Continue to add 2 ml of freshly prepared *N*-(1-naphthyl)ethylenediamine hydrochloride/propylene glycol TS, allow to stand in a water-bath at 22–25 °C for exactly 10 minutes, and dilute to volume with water.

Proceed immediately with the measurement of the absorbances of solutions A and B against the blank solution at a wavelength of about 465 nm. The absorbance of solution A does not exceed that of solution B.

Assay. Carry out the combustion as described under 2.4 Oxygen flask method, but using about 4 mg of Iotroxic acid, accurately weighed, and allowing the absorbing liquid after rinsing to stand for 20–30 minutes. Titrate the liberated iodine with sodium thiosulfate (0.02 mol/l) VS.

Each ml of sodium thiosulfate (0.02 mol/l) VS is equivalent to 0.6754 mg of $C_{22}H_{18}I_6N_2O_9$.

Additional requirement for Iotroxic acid for parenteral use

Complies with the monograph for "Parenteral preparations".

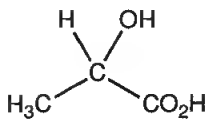
Pyrogens. Carry out the test as described under 3.5 Test for pyrogens, injecting, per kg of the rabbit's mass, a solution in sterile water R containing 0.6 g of Iotroxic acid in not more than 5 ml.

Additional requirement for Iotroxic acid for sterile use

Complies with 3.2.1 Test for sterility of non-injectable preparations.

ACIDUM LACTICUM

LACTIC ACID



$C_3H_6O_3$

Relative molecular mass. 90.08

Chemical name. Lactic acid; 2-hydroxypropanoic acid; CAS Reg. No. 50-21-5.

Description. A colourless or slightly yellow, clear, syrupy, caustic liquid; odourless or with a slight characteristic odour.

Miscibility. Miscible with water, ethanol (~750 g/l) TS, and ether R.

Category. Used in the preparation of sodium lactate solution as electrolyte.

Storage. Lactic acid should be kept in a tightly closed container.

Additional information. Lactic acid as described is not suitable for parenteral administration (haemodialysis). Lactic acid is usually a racemate (*RS*), but the (+)-(*S*)-isomer may predominate; it is hygroscopic.

Requirements

Definition. Lactic acid is a mixture of lactic acid, its condensation products, and water, the equilibrium between the components being dependent on the concentration and temperature.

Lactic acid contains not less than **88.0% *m/m*** and not more than the equivalent of **92.0% *m/m*** of $C_3H_6O_3$.

Identity tests

A. To 5 ml of a solution containing 5 mg of Lactic acid, add 1 ml of bromine TS1 and 0.5 ml of sulfuric acid (~100 g/l) TS and heat on a water-bath until the colour is discharged, while stirring occasionally with a glass rod (an odour of acetaldehyde is perceptible). Add 4 g of ammonium sulfate R, mix, and add, drop by drop without mixing, 0.2 ml of a solution containing 10 mg of sodium nitroprusside R per ml of sulfuric acid (~100 g/l) TS. Without prior mixing add 1 ml of ammonia (~260 g/l) TS and allow to stand for 30 minutes; a dark green ring is produced at the interface of the two liquids.

B. A mixture of 1 ml and 9 ml of water shows an acid reaction with pH-indicator paper R.

C. Relative density, $d_{20}^{20} = 1.20 - 1.21$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Iron. Use 1.0 g; the solution complies with the 2.2.4 Limit test for iron; not more than 40 µg/g.

Calcium. Dissolve 5 g in 42 ml of sodium hydroxide (1 mol/l) VS and dilute to 50 ml with distilled water. Dilute 5 ml to 15 ml with distilled water. (Keep the remaining solution for the "Chlorides" and "Sugars and other reducing substances" tests.)

To 0,2 ml of ethanolic calcium standard (100 µg/ml Ca) TS add 1 ml of ammonium oxalate (50 g/l) TS and allow to stand for 1 minute. Add 1 ml of acetic acid (~60 g/l) TS and the above-prepared 15 ml of test solution. Similarly, prepare a reference solution but using 10 ml of calcium standard (10 µg/ml) TS and 5 ml of water. Allow both solutions to stand for 15 minutes. Any opalescence observed in the test solution is not more intense than that of the reference solution (200 µg/g).

Chlorides. Dissolve 0.1 g in 10 ml of water, acidify with nitric acid (~130 g/l) TS, and add a few drops of silver nitrate (40 g/l) TS; no opalescence is immediately produced.

Sulfates. Take 25 ml of the solution prepared for the "Calcium" test and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 200 µg/g.

Sugars and other reducing substances. To 1 ml of the solution prepared for the "Calcium" test add 1 ml of hydrochloric acid (1 mol/l) VS, heat to boiling, cool, and add 1.5 ml of sodium hydroxide (1 mol/l) VS and 2 ml of potassiocupric tartrate TS. Heat to boiling; no red or greenish precipitate is produced.

Volatile fatty acids. Heat 5 g cautiously in a glass-stoppered flask at 50 °C for 10 minutes; on opening of the flask no unpleasant odour resembling that of the lower fatty acids is noticed.

Methanol and methyl esters. Place 2 g in a round-bottomed flask and add 10 ml of water. Cool in ice, add cautiously a mixture of 7.5 ml of water with 22.5 ml of potassium hydroxide (~400 g/l) TS, and cool in ice for a further 10–15 minutes. Connect to a suitable condenser and steam distil. Collect the distillate in a 10-ml graduated flask containing 1 ml of ethanol (~750 g/l) TS and distil until a volume of at least 9.5 ml is obtained. Dilute to 10 ml with water and to 1 ml add 5 ml of potassium permanganate/phosphoric acid TS and mix. After 15 minutes add 2 ml of oxalic acid/sulfuric acid TS, stir with a glass rod until the solution is colourless, and then add 5 ml of decolorized fuchsin TS. Allow to stand for 2 hours. The solution is not more intensely coloured than a reference solution prepared similarly, but using instead of the distillate 1.0 ml of a solution containing 100 µg of methanol R and 0.1 ml of ethanol (~750 g/l) TS per ml (500 µg/g of methanol).

Citric, oxalic, phosphoric, and tartaric acid. To 1 g dissolved in 10 ml of water add 40 ml of calcium hydroxide TS and boil for 2 minutes; no turbidity is produced.

Ether-insoluble substances. Dissolve 1 g in 25 ml of ether R and compare it with 25 ml of ether R; both solutions are equally clear.

Colour. Lactic acid is not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Assay. Place about 1 g, accurately weighed, in a glass-stoppered flask, and add 10 ml of water and 20 ml of sodium hydroxide (1 mol/l) VS. Stopper the flask and allow to stand for 30 minutes. Back-titrate with hydrochloric acid (1 mol/l) VS, using 0.5 ml of phenolphthalein/ethanol TS as indicator.

Each ml of sodium hydroxide (1 mol/l) VS is equivalent to 90.08 mg of $C_3H_6O_3$.

Additional requirements for Lactic acid for parenteral use

Complies with the monograph for "Parenteral preparations".

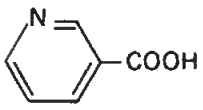
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 83.3 IU of endotoxin RS per mg.

ACIDUM NICOTINICUM
NICOTINIC ACID

Molecular formula. $C_6H_5NO_2$

Relative molecular mass. 123.1

Graphic formula.



Chemical name. 3-Pyridinecarboxylic acid; CAS Reg. No. 59-67-6.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; freely soluble in boiling water; soluble in 100 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Component of vitamin B complex; vasodilator.

Storage. Nicotinic acid should be kept in a well-closed container, protected from light.

Requirements

Definition. Nicotinic acid contains not less than 99.0% and not more than 101.0% of $C_6H_5NO_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or all 3 tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nicotinic acid RS or with the *reference spectrum* of nicotinic acid.

B. Heat 0.1 g with 0.4 g of anhydrous sodium carbonate R; pyridine, perceptible by its odour, is produced.

C. Dissolve 10 mg in 10 ml of water. To 2 ml add 2 ml of thiocyanate reagent, obtained by adding, drop by drop, ammonium thiocyanate (0.1 mol/l) VS to bromine TS1 until the yellow coloration disappears. Then add 3 ml of aniline (25 g/l) TS and shake; a yellow colour is produced.

D. Melting temperature, about 235 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 1.25 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, filter if necessary, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 13 mg/ml solution, 3.0–3.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 85 volumes of 1-propanol R, 10 volumes of anhydrous formic acid R, and 5 volumes of water as the mobile phase. For the test solution, dissolve 75 mg in 5.0 ml of water with gentle heating; this constitutes solution A. Prepare a reference solution containing 0.12 mg/ml of nicotinic acid RS; this constitutes solution B. Apply to the plate 10 µl of solution A using two 5-µl aliquots, allowing the plate to dry in a current of cold air after the first application; then apply separately 5 µl of solution B. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and examine the chromatogram in ultraviolet light (254 nm). Beside the principal spot, not more than 3 spots are obtained with solution A, and they are not more intense than the spot obtained with solution B.

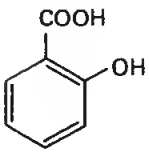
Assay. Dissolve about 0.25 g, accurately weighed, in 50 ml of carbon-dioxide-free water R, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 12.31 mg of $C_6H_5NO_2$.

ACIDUM SALICYLICUM
SALICYLIC ACID

Molecular formula. $C_7H_6O_3$

Relative molecular mass. 138.1

Graphic formula.



Chemical name. 2-Hydroxybenzoic acid; CAS Reg. No. 69-72-7.

Description. Colourless crystals, usually needle-like, or a white, crystalline powder; odourless.

Solubility. Slightly soluble in water; soluble in 4 parts of ethanol (~750 g/l) TS and in 3 parts of ether R.

Category. Keratolytic.

Storage. Salicylic acid should be kept in a well-closed container.

Requirements

Definition. Salicylic acid contains not less than 99.0% and not more than 101.0% of $C_7H_6O_3$, calculated with reference to the dried substance.

Identity test
Dissolve 0.14 g in 1 ml of sodium hydroxide (1 mol/l) VS and add 5 ml of water; this solution yields the reaction described under 2.1 General identification tests as characteristic of salicylates.

Melting range. 158–161 °C.

Heavy metals. Use 2.0 g and 15 ml of ethanol (~750 g/l) TS for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 2; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 1.7 g in 40 ml of boiling water, cool and filter. Add 2 ml of nitric acid (~130 g/l) TS to the filtrate and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.15 mg/g.

Sulfates. Dissolve 2.5 g in 40 ml of boiling water, cool, filter, and proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Solution in ethanol. A solution of 1.0 g in 10 ml of ethanol (~750 g/l) TS is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight over silica gel, desiccant, R at ambient temperature; it loses not more than 5.0 mg/g.

Assay. Dissolve about 0.3 g, accurately weighed, in 15 ml of neutralized ethanol TS and add 20 ml of water. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 13.81 mg of $C_7H_6O_3$.

ADEPS LANAE
WOOL FAT

Adeps lanae cum aqua
Hydrous wool fat

Chemical name. Lanolin; CAS Reg. No. 8020-84-6.

Other name. Anhydrous lanolin, lanolin. (In certain countries the name lanolin is used to describe a formulation containing wool fat, water, and liquid paraffin.)

Description. Wool fat is a brown-yellow, unctuous mass. Hydrous wool fat is a yellowish white, unctuous mass. Odour, characteristic.

Solubility. Practically insoluble in water; soluble in ether R; slightly soluble in boiling ethanol (~750 g/l) TS.

Category. Ointment base.

Storage. Wool fat should be kept in a well-closed container.

Additional information. Melted Wool fat is a yellow, clear or almost clear liquid. On heating, Hydrous wool fat first separates into two layers, water is then driven off, and a transparent residue is formed; after cooling, a yellowish, tenacious, soft mass is produced. Melting point for Wool fat and Hydrous wool fat after drying (use the residue from the "Wool fat content"), 36–44 °C.

Requirements

Definition. Wool fat is a purified wax-like material obtained from the raw wool of sheep (*Ovis aries* L).

Hydrous wool fat is a mixture of 75% *m/m* of wool fat and 25% *m/m* of water.

Identity tests

A. Dissolve 0.5 g in 5 ml of chloroform R and add 1 ml of acetic anhydride R and 0.1 ml of sulfuric acid (~1760 g/l) TS; a green colour is produced.

B. Dissolve 0.5 g in 5 ml of chloroform R and carefully superimpose 5 ml of sulfuric acid (~1760 g/l) TS; a bright, brown-red ring is gradually formed at the interface of the two liquids.

Acid value. Wool fat, not more than 1.0; Hydrous wool fat, not more than 0.8.

Saponification value. Reflux for 4 hours; Wool fat, 90–105; Hydrous wool fat, 67–79.

Sulfated ash. Wool fat, not more than 1.5 mg/g; Hydrous wool fat, not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 1 hour; Wool fat loses not more than 5.0 mg/g; Hydrous wool fat loses not more than 0.32 g/g.

Wool fat content. Heat 30 g of Hydrous wool fat to constant mass on a water-bath, stirring continuously, and weigh; the residue weighs between 21.8 g and 23.3 g (72.5–77.5% *m/m*). (Keep the residue for "Water-absorption capacity", "Paraffins", and the melting point in "Additional information".)

Water-absorption capacity. Place 10 g of Wool fat or Hydrous wool fat after drying (use the residue from "Wool fat content") in a mortar. Using a burette, add water in portions of 0.2–0.5 ml, stirring vigorously after each addition to incorporate the water, until visible droplets separate and cannot be absorbed; not less than 20 ml of water is absorbed.

Water-soluble acid and alkaline substances. Melt 5 g of Wool fat or 6.7 g of Hydrous wool fat on a water-bath, add 75 ml of water heated to 90–95 °C, and shake vigorously for 2 minutes. Cool and filter through a filter-paper previously moistened with water. To 60 ml of the filtrate, which may show some cloudiness (keep the remaining filtrate for "Water-soluble oxidizable substances" and "Ammonia"), add 0.25 ml of bromothymol blue/ethanol TS; not more than 0.2 ml of hydrochloric acid (0.02 mol/l) VS or 0.15 ml of sodium hydroxide (0.02 mol/l) VS is required to change the colour of the indicator (blue-yellow).

Water-soluble oxidizable substances. To 10 ml of the filtrate retained in the above test add 1 ml of sulfuric acid (~100 g/l) TS and 0.1 ml of potassium permanganate (0.02 mol/l) VS, and allow to stand for 10 minutes; the colour is not completely discharged.

Paraffins. To 40 ml of dehydrated ethanol R add 0.5 g of Wool fat or Hydrous wool fat after drying (use the residue from "Wool fat content") and boil; the solution is clear or not more than opalescent.

Ammonia. To 5 ml of the filtrate from "Water-soluble acid and alkaline substances" add 0.5 ml of sodium hydroxide (1 mol/l) VS and boil; the vapours do not turn red litmus paper R to blue.

ADEPS SOLIDUS
HARD FAT

Chemical name. Hard fat.

Description. A white, brittle, unctuous mass; almost odourless.

Solubility. Practically insoluble in water; freely soluble in ether R; slightly soluble in ethanol (~750 g/l) TS.

Category. Suppository base.

Storage. Hard fat should be kept in a well-closed container, protected from light, and stored at a temperature not exceeding 5 °C below the melting range.

Additional information. On warming, hard fat melts to a colourless to slightly yellow liquid. On shaking the molten material with an equal quantity of hot water, forms a white emulsion.

Note: For certain applications, it may be necessary to comply with more restrictive specifications.

Requirements

Definition. Hard fat is a mixture of mono-, di- and triglyceride esters of higher saturated fatty acids ($C_{10}H_{20}O_2$ to $C_{18}H_{36}O_2$).

Melting range. Cool the charged tube at a temperature below 10 °C for 24 hours; 33–40 °C.

Acid value. Not more than 0.5.

Hydroxyl value. Not more than 50.

Iodine value. Not more than 3.

Peroxide value. Not more than 6.

Saponification value. Use 2.0 g; 220–250.

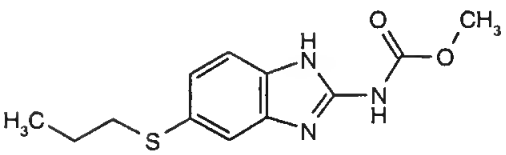
Unsaponifiable matter. Use 5 g; not more than 1.0%.

Ash. Not more than 0.5 mg/g.

Alkaline impurities. Dissolve 2 g in a mixture of 1.5 ml of ethanol (~750 g/l) TS and 3 ml of ether R. Add 0.05 ml of bromophenol blue/ethanol TS; not more than 0.15 ml of hydrochloric acid (0.01 mol/l) VS is required to change the colour of the solution to yellow.

Decomposition products. Shake 1 g with 1 ml of hydrochloric acid (~420 g/l) TS for 1 minute, add 1 ml of resorcinol/toluene TS, shake for 5 seconds, and allow to stand for 5 minutes; the aqueous layer is not more intensely coloured than 1 ml of a mixture of 0.4 ml of potassium permanganate (0.002 mol/l) VS and 9.6 ml of water.

ALBENDAZOLUM
ALBENDAZOLE



$C_{12}H_{15}N_3O_2S$

Relative molecular mass. 265.3

Chemical name. Methyl 5-(propylthio)-2-benzimidazolecarbamate; CAS Reg. No. 54965-21-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water; soluble in glacial acetic acid R; slightly soluble in acetone R, very slightly soluble in ethanol (~750 g/l) TS.

Category. Anthelminthic.

Storage. Albendazole should be kept in a well-closed container, protected from light.

Additional information. Melting temperature, about 210 °C, with decomposition.

Requirements

Albendazole contains not less than **98.0%** and not more than **101.0%** of $C_{12}H_{15}N_3O_2S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from albendazole RS or with the *reference spectrum* of albendazole.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Ignite about 0.1 g; fumes are evolved, staining lead acetate paper R black.

D. Add about 0.1 g to 3 ml of sulfuric acid (~100 g/l) TS and warm to dissolve. Add about 1 ml of potassium iodobismuthate/acetic acid TS; a reddish brown precipitate is produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 4 hours; it loses not more than 5.0 mg/g.

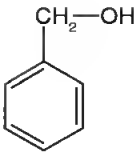
Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 6 volumes of dichloromethane R, 1 volume of ether R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 5 solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of anhydrous formic acid R containing (A) 10.0 mg of Albendazole per ml, (B) 1.0 mg of Albendazole per ml, (C) 1.0 mg of albendazole RS per ml, (D) 0.05 mg of albendazole RS per ml, and (E) 0.025 mg of albendazole RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than the principal spot obtained with solution D (0.5%), and only one spot may be more intense than the principal spot obtained with solution E (0.25%).

Assay. Dissolve about 0.25 g, accurately weighed, in 3 ml of anhydrous formic acid R, and add 40 ml of glacial acetic acid R1. Then add 0.2 ml of 1-naphthol-benzein/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS until a green colour is obtained as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.53 mg of $C_{12}H_{15}N_3O_2S$.

ALCOHOL BENZYLICUS
BENZYL ALCOHOL



C_7H_8O

Relative molecular mass. 108.1

Chemical name. Benzyl alcohol; benzenemethanol; CAS Reg. No. 100-51-6.

Description. A clear, colourless, oily liquid; odour, slightly aromatic.

Solubility. Soluble in water; miscible with ethanol (~750 g/l) TS, ether R, fatty and essential oils.

Category. Antimicrobial preservative.

Storage. Benzyl alcohol should be kept in a tightly closed container, protected from light.

Additional information. Benzyl alcohol is affected by air and light, and should be protected from exposure to excessive heat.

Requirements

Identity test
Add 2–3 drops to 5 ml of potassium permanganate (25 g/l) TS, and acidify with 1 ml of sulfuric acid (~100 g/l) TS; an odour of benzaldehyde is perceptible.

Refractive index. $n_D^{20} = 1.538 - 1.541$.

Relative density. $d_{20}^{20} = 1.043 - 1.050$.

Clarity of solution. Shake 2 ml with 60 ml of water; the solution is clear.

Sulfated ash. Evaporate 10 ml from a porcelain crucible and ignite to constant mass; not more than 0.05 mg/g.

Acidity. To 10 ml add 10 ml of ethanol (~750 g/l) TS and 1 ml of phenolphthalein/ethanol TS; not more than 1 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is required to obtain the midpoint of the indicator (pink).

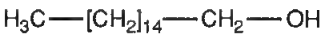
Peroxide value. Not more than 5.

Chlorinated compounds. Mix 2 g with 50 ml of amyl alcohol R using a dry flask, add in small quantities 3 g of sodium R (*Note*: Proceed with caution), connect the flask to a reflux condenser, warm gently until the evolution of hydrogen ceases, and boil gently for 1 hour. Cool the liquid to just below 100 °C and add 50 ml of water, 5 ml of silver nitrate (0.1 mol/l) VS, and 20 ml of nitric acid (~1000 g/l) TS. Titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the procedure without the Benzyl alcohol being examined and make any necessary corrections. The difference between the titrations does not exceed 0.3 ml.

Aldehydes. Transfer about 20 ml, accurately measured, to a 250-ml conical flask containing 5 ml of a solution containing 3.5 g of hydroxylamine hydrochloride R in 100 ml of ethanol (~600 g/l) TS, add 50 ml of ethanol (~600 g/l) TS, and mix. Allow to stand for 10 minutes, add 1 ml of bromophenol blue/ethanol TS, and titrate with sodium hydroxide (0.1 mol/l) VS to a light green endpoint. Repeat the procedure without the Benzyl alcohol being examined and make any necessary corrections.

The net volume of sodium hydroxide (0.1 mol/l) VS consumed does not exceed 4.0 ml, corresponding to 2.0 mg/g of benzaldehyde. Benzyl alcohol used for parenteral administration does not consume more than 1.0 ml, corresponding to 0.5 mg/g of benzaldehyde.

ALCOHOL CETYLICUS
CETYL ALCOHOL



$C_{16}H_{34}O$

Chemical name. 1-Hexadecanol; CAS Reg. No. 36653-82-4.

Description. Unctuous, colourless flakes or a white, crystalline mass; odour, faint and characteristic.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS and ether R.

Category. Emulsifying agent; viscosity-increasing agent.

Storage. Cetyl alcohol should be kept in a well-closed container.

Requirements

Definition. Cetyl alcohol is a mixture of solid alcohols consisting mainly of 1-hexadecanol ($C_{16}H_{34}O$).

Melting range. 46–51 °C.

Acid value. Not more than 2.

Saponification value. Not more than 2.

Iodine value. Not more than 3.

Hydroxyl value. Place about 2 g, accurately weighed, in a glass-stoppered 250-ml flask, and add 2 ml of pyridine R and 10 ml of toluene R. To this mixture add 10 ml of a solution of acetyl chloride prepared by adding 10 ml of acetyl chloride R to 90 ml of toluene R. Insert the stopper in the flask, and heat in a water-bath at about 65 °C for 20 minutes. Add 25 ml of water, stopper the flask, and shake vigorously for several minutes to decompose the excess acetyl chloride. Titrate while shaking the flask vigorously throughout the titration in order to maintain the contents in an emulsified condition with carbonate-free sodium hydroxide (1 mol/l) VS, using 0.5 ml of phenolphthalein/ethanol TS as indicator, to a permanent pink endpoint. Repeat the procedure without the Cetyl alcohol being examined and make any necessary corrections.

Multiply the difference in ml between the two titrations of carbonate-free sodium hydroxide (1 mol/l) VS by 56.1 and divide it by the mass in g of Cetyl alcohol used; 218–238.

Paraffin. Dissolve 0.5 g in 20 ml of neutralized ethanol TS by warming; the solution is clear and not more intensely coloured than standard colour solution Bn2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

ALCOHOL CETYLSTEARYLICUS
CETOSTEARYL ALCOHOL

Chemical name. 1-Octadecanol mixture with 1-hexadecanol; CAS Reg. No. 67762-27-0.

Description. A white or yellowish white, unctuous mass, or white flakes or granules; odour, characteristic and faint.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS and ether R.

Category. Retarding agent; stiffening agent.

Storage. Cetostearyl alcohol should be kept in a well-closed container, protected from light.

Requirements

Definition. Cetostearyl alcohol is a mixture of solid aliphatic alcohols consisting mainly of cetyl alcohol and stearyl alcohol.

Melting range. 43–53 °C.

Acid value. Not more than 2.

Saponification value. Not more than 2.

Iodine value. Not more than 4.

Hydroxyl value. Place 2.0 g in a glass-stoppered 250-ml flask, and add 2 ml of pyridine R and 10 ml of toluene R. To this mixture add 10 ml of a solution of acetyl chloride prepared by adding 10 ml of acetyl chloride R to 90 ml of toluene R. Insert the stopper in the flask, and heat in a water-bath at about 65 °C for 20 minutes. Add 25 ml of water, stopper the flask, and shake vigorously for several minutes to decompose the excess acetyl chloride. Titrate while shaking the flask vigorously throughout the titration in order to maintain the contents in an emulsified condition with carbonate-free sodium hydroxide (1 mol/l) VS, using 0.5 ml of phenolphthalein/ethanol TS as indicator, to a permanent pink endpoint. Repeat the procedure without the Cetostearyl alcohol being examined and make any necessary corrections.

Multiply the difference in ml between the two titrations of carbonate-free sodium hydroxide (1 mol/l) VS by 56.1 and divide it by the mass in g of Cetostearyl alcohol used; 208–228.

Paraffin. Dissolve 0.5 g in 20 ml of neutralized ethanol TS by warming; the solution is clear and not more intensely coloured than standard colour solution Bn2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

ALCOHOLUM
ALCOHOL

Description. A clear, colourless, and mobile liquid; odour, characteristic.

Miscibility. Miscible with water, ether R, and glycerol R.

Category. Solvent; antiseptic.

Storage. Alcohol should be kept in a well-closed container, and stored whenever possible at a temperature between 8 and 15 °C.

Labelling. The designation on the container should state the content of Alcohol in % *v/v*.

Additional information. Different concentrations of Alcohol are prepared from alcohol 96% *v/v* and water at about 20 °C.

Note: Contraction of volume and rise in temperature occur when mixing Alcohol with water.

Alcohol		**Approximate relative density** d_{20}^{20}	**Volume of Alcohol (ml) 96% *v/v* to be diluted to 1000 ml with water**
% *v/v*	**g/l**		
90	701.4	0.8304	934
80	625.3	0.8610	831
70	561.8	0.8872	727
60	488.0	0.9109	623
50	404.6	0.9320	519
45	341.3	0.9412	468
25	209.0	0.9699	259
20	163.8	0.9754	207

Requirements

Alcohol is a mixture of ethanol and water. It contains not less than **98.0% *v/v*** and not more than the equivalent of **102.0% *v/v*** of the declared content of ethanol (C_2H_6O).

Identity tests

A. To 0.25 ml in a small beaker add 1 ml of potassium permanganate (10 g/l) TS and 0.5 ml of sulfuric acid (0.5 mol/l) VS; cover the beaker immediately with a filter-paper moistened with a recently prepared solution of 0.1 g of sodium nitroprusside R and 0.5 g of piperazine hydrate R in 5 ml of water; a dark blue colour is produced on the filter-paper, which fades after a few minutes.

B. To a few drops add 1 ml of sulfuric acid (~1760 g/l) TS and a few drops of potassium dichromate (100 g/l) TS; a green colour is developed and an odour of acetaldehyde is perceptible.

Relative density. According to the labelled concentration, relate to the value stated under "Additional information".

Non-volatile residue. Place 100 ml in a porcelain dish and heat on a water-bath until volatilized, dry the residue at 105 °C for 1 hour, and weigh; not more than 5 mg.

Water-insoluble substances. Dilute a volume of Alcohol with an equal volume of water; the mixture is clear and, after cooling to 10 °C, remains clear for 30 minutes.

Acidity. Add 20 ml of carbon-dioxide-free water R and 3 drops of phenolphthalein/ethanol TS to 20 ml of Alcohol; the colour remains unchanged. Titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS; not more than 0.5 ml is required to obtain the midpoint of the indicator (pink).

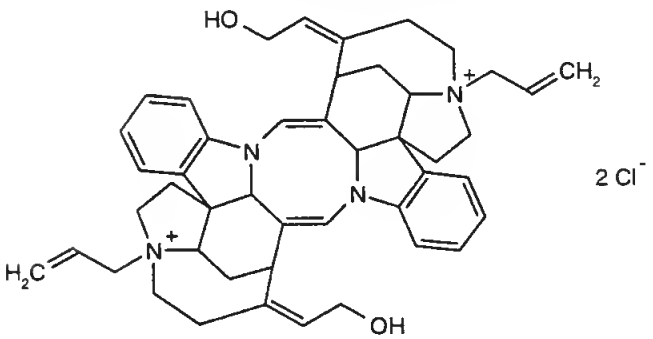
Aldehydes and other foreign organic substances. Thoroughly clean a glass-stoppered cylinder with hydrochloric acid (~250 g/l) TS, rinse with water and the Alcohol to be examined. Place 20 ml of Alcohol in the cylinder. Cool the contents to about 15 °C and, by means of a carefully cleaned pipette and noting the time, add 0.1 ml of potassium permanganate (0.02 mol/l) VS. Mix at once by inverting the stoppered cylinder, and allow to stand at 15 °C for exactly 5 minutes; the pink colour does not entirely disappear.

Fusel oil and allied impurities. Carefully protected from dust, allow 25 ml to evaporate spontaneously from a porcelain dish until it is barely moist; no foreign odour is perceptible. Add a few drops of sulfuric acid (~1760 g/l) TS; the colour does not change to brown or red.

Methanol. To 1 drop add 1 drop of water, 1 drop of phosphoric acid (~105 g/l) TS, and 2 drops of potassium permanganate (25 g/l) TS, mix, and allow to stand for 1 minute. Add, drop by drop, sodium metabisulfite (50 g/l) TS until the permanganate colour is discharged. If a brown colour remains, add 1 drop of phosphoric acid (~105 g/l) TS. To the colourless solution, add 5 ml of freshly prepared disodium chromotropate TS, and heat on a water-bath at 60 °C for 10 minutes; the colour does not change to violet.

Benzene. Record an absorption spectrum of the Alcohol in a 1-cm layer against water between 220 nm and 350 nm. The absorbance at about 220 nm is not more than 0.30, at about 230 nm not more than 0.18, at about 240 nm not more than 0.08, and between 270 and 350 nm not more than 0.02. A curve drawn through these points is smooth.

ALCURONII CHLORIDUM
ALCURONIUM CHLORIDE



$C_{44}H_{50}Cl_2N_4O_2$

Relative molecular mass. 737.8

Chemical name. *N,N'*-Diallylnortoxiferinium dichloride; CAS Reg. No. 15180-03-7.

Other name. Alcuronium dichloride.

Description. A white to yellow-white, crystalline powder.

Solubility. Soluble in water and ethanol (~750 g/l) TS.

Category. Muscle relaxant.

Storage. Alcuronium chloride should be kept in a tightly closed container.

Labelling. The designation Alcuronium chloride for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. *CAUTION*: Alcuronium chloride is highly toxic. It must be handled with care, avoiding contact with the skin and inhalation of airborne particles. It is hygroscopic.

Requirements

Alcuronium chloride contains not less than **98.0%** and not more than **101.0%** of $C_{44}H_{50}Cl_2N_4O_2$, calculated with reference to the anhydrous substance.

Note: All tests must be carried out immediately after opening the container, and as rapidly as possible.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from alcuronium chloride RS or with the *reference spectrum* of alcuronium chloride.

B. The absorption spectrum of a 14 µg/ml solution in phosphate buffer, pH 7.0 (0.067 mol/l) TS, when observed between 230 nm and 350 nm, exhibits a maximum at about 293 nm and a minimum at about 237 nm; the absorbance of a 1-cm layer at the maximum wavelength is about 0.9.

C. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 10 mg/ml solution, measured within 10 minutes of preparation, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = -430\,°$ to $-451\,°$.

Heavy metals. Use 2.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method B; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.10 g in 10 ml of carbondioxide- free water R is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 0.050 g/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 6.0–8.5.

Related substances. Carry out the test protected from daylight until the start of detection as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 1 volume of methanol R and 1 volume of ammonium nitrate TS as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions in methanol R containing (A) 40 mg of Alcuronium chloride per ml, (B) 40 mg of alcuronium chloride RS per ml, (C) 0.20 mg of alcuronium chloride RS per ml, and (D) 0.10 mg of alcuronium chloride RS per ml. Prior to development allow the plate to dry in a current of cold air and place in a chromatographic chamber. After removing the plate from the chromatographic chamber, allow it again to dry in a current of cold air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%), and no more than 3 of these spots are greater than the spot obtained with solution D (0.25%).

Assay. To about 0.3 g, accurately weighed, add 70 ml of acetic anhydride R and place the mixture in an ultrasonic bath for 15 seconds. Titrate the turbid solution with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.89 mg of $C_{44}H_{50}Cl_2N_4O_2$.

Additional requirements for Alcuronium chloride for parenteral use

Complies with the monograph for "Parenteral preparations".

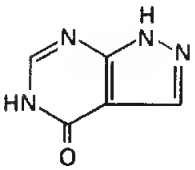
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 17.5 IU of endotoxin RS per mg.

ALLOPURINOLUM
ALLOPURINOL

Molecular formula. $C_5H_4N_4O$

Relative molecular mass. 136.1

Graphic formula.



Chemical name. 1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one; 1-*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol; CAS Reg. No. 315-30-0.

Description. A white or almost white, microcrystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water and in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Xanthine oxidase inhibitor.

Storage. Allopurinol should be kept in a well-closed container.

Requirements

Definition. Allopurinol contains not less than 98.0% and not more than 101.0% of $C_5H_4N_4O$, calculated with reference to the dried substance.

Identity test

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from allopurinol RS or with the *reference spectrum* of allopurinol.

B. Dissolve 0.1 g in 10 ml of sodium hydroxide (0.1 mol/l) VS and add sufficient hydrochloric acid (0.1 mol/l) VS to produce 100 ml; dilute 10 ml to 100 ml with hydrochloric acid (0.1 mol/l) VS and dilute 10 ml of this solution again to 100 ml with hydrochloric acid (0.1 mol/l) VS. The absorption spectrum of the resulting solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 250 nm and a minimum at about 231 nm. The

absorbance at the maximum wavelength is about 0.55. The ratio of the absorbance of a 1-cm layer at 231 nm to that at 250 nm is between 0.52 and 0.62.

C. Dissolve 0.05 g in 5 ml of sodium hydroxide (~80 g/l) TS, add 1 ml of alkaline potassio-mercuric iodide TS, heat to boiling, and allow to stand; a yellow, flocculent precipitate is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R3 as the coating substance. Prepare the mobile phase by shaking 200 ml of 1-butanol R with 200 ml of ammonia (~100 g/l) TS. Apply separately to the plate 10 µl of each of 2 freshly prepared solutions in diethylamine R containing (A) 25 mg of the test substance per ml and (B) 0.050 mg of 3-aminopyrazole-4-carboxamide hemisulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.25 g, accurately weighed, in 50 ml of dimethylformamide R, add 2 drops of thymol blue/dimethylformamide TS and titrate with sodium methoxide (0.1 mol/l) VS to a blue endpoint, as described under 2.6 Non-aqueous titration, Method B. Each ml of sodium methoxide (0.1 mol/l) VS is equivalent to 13.61 mg of $C_5H_4N_4O$.

ALUMINII HYDROXIDUM
ALUMINIUM HYDROXIDE

Molecular formula. $Al(OH)_3$

Relative molecular mass. 78.00

Chemical name. Aluminium hydroxide; CAS Reg. No. 21645-51-2.

Description. A white, fine, amorphous powder; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in hydrochloric acid (~70 g/l) TS and sodium hydroxide (~80 g/l) TS.

Category. Antacid.

Storage. Aluminium hydroxide should be kept in a tightly closed container.

Requirements

Definition. Aluminium hydroxide contains not less than 71.9% and not more than 94.9% of $Al(OH)_3$.

Identity test
Dissolve 0.10 g by heating in 5 ml of sodium hydroxide (~80 g/l) TS. To the clear solution add 0.5 g of ammonium chloride R; a white, gelatinous precipitate is produced.

Heavy metals. For the preparation of the test solution dissolve 0.5 g, while heating, in 5 ml of acetic acid (~300 g/l) TS, dilute to 10 ml with water and filter. Adjust the pH of the filtrate to 3–4, dilute to 40 ml with water and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 60 µg/g.

Arsenic. Use a solution of 3.3 g in 20 ml of sulfuric acid (~100 g/l) TS and 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 5 µg/g.

Ammonium salts. Transfer 5.0 g to an ammonia distillation apparatus, add 25 ml of sodium hydroxide (~200 g/l) TS and 200 ml of water, distil about 100 ml, collecting the distillate in 25.0 ml of hydrochloric acid (0.1 mol/l) VS.

Titrate the excess acid with sodium hydroxide (0.1 mol/l) VS using methyl red/ethanol TS as indicator; not less than 22.5 ml of sodium hydroxide (0.1 mol/l) VS is required.

Chlorides. Dissolve 0.10 g in 2 ml of nitric acid (~130 g/l) TS, boil, cool, dilute to 10 ml with water and filter. Proceed with 5 ml of the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 10 mg/g.

Sulfates. Dissolve 0.10 g in 5 ml of hydrochloric acid (~70 g/l) TS, boil, cool, dilute to 10 ml with water and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 5 mg/g.

Neutralizing capacity. Pass a sufficient quantity of the test substance, triturated if necessary, through a sieve of nominal mesh aperture 150 µm, add 0.50 g to 200 ml of hydrochloric acid (0.05 mol/l) VS previously heated to 37 °C, and stir continuously, maintaining the temperature at 37 °C; the pH of the solution at 37 °C, after 10, 15, and 20 minutes, is not less than 1.8, 2.3, and 3.0, respectively, and at no time more than 4.0. Add 10 ml of hydrochloric acid (0.5 mol/l) VS previously heated to 37 °C, stir continuously for 1 hour, maintaining the temperature at 37 °C. Titrate the solution with sodium hydroxide (0.1 mol/l) VS to pH 3.5. The neutralizing capacity is not less than 83.3% of the theoretical amount when calculated by the formula $(1000)(150\text{-}y)/A \times W \times 38.46$, in which y is the number of ml of sodium hydroxide (0.1 mol/l) VS required, A is the percent of $Al(OH)_3$ obtained in the assay, W is the quantity, in g, of test substance taken, and 38.46 is the theoretical value of each g of $Al(OH)_3$.

Alkaline impurities. The pH of a 0.04 g/ml suspension in carbon-dioxide-free water R is not more than 10.0.

Assay. Proceed with about 0.15 g, accurately weighed, as described under 2.5 Complexometric titrations for aluminium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 3.900 mg of $Al(OH)_3$.

ALUMINII MAGNESII SILICAS
ALUMINIUM MAGNESIUM SILICATE

Chemical name. Magnesium aluminosilicate; aluminium magnesium silicate; CAS Reg. No. 1327-43-1.

Other name. Aluminum magnesium silicate.

Description. A creamy white or greyish white powder or flakes; odourless or almost odourless.

Solubility. Practically insoluble in water and most organic solvents; when added to water it swells to form a colloidal suspension.

Category. Suspending agent; viscosity-increasing agent; tabletting aid.

Storage. Aluminium magnesium silicate should be kept in a well-closed container.

Additional information. Several types of aluminium magnesium silicate occur, of which the powder or flakes vary in shape and size.

Requirements

Definition. Aluminium magnesium silicate is a natural, colloidal hydrated aluminium magnesium silicate, a saponite, freed from gritty particles.

Identity tests

A. In a metal crucible fuse 1 g with 2 g of anhydrous sodium carbonate R. To the fused mass, add hot water and filter. (Keep the filtrate for test B.) To the residue remaining on the filter, add 5 ml of hydrochloric acid (~70 g/l) TS and 10 ml of water, and filter. To the filtrate add 2 ml of ammonia (~100 g/l) TS; a white, gelatinous precipitate is produced. Centrifuge (keep the precipitate for test C), neutralize 2 ml of the supernatant liquid, add 0.2 ml of titan yellow TS and 0.5 ml of sodium hydroxide (0.1 mol/l) VS; a bright red turbidity is formed which gradually settles to give a bright red precipitate.

B. Acidify the filtrate from test A with hydrochloric acid (~420 g/l) TS and evaporate to dryness. Heat the residue with a mixture of 10 mg of calcium fluoride R and a few drops of sulfuric acid (~1760 g/l) TS; a gas is evolved. Add a few ml of water; it gives a white precipitate.

C. Dissolve the precipitate from test A in 2 ml of hydrochloric acid (~70 g/l) TS and add 0.5 ml of alkaline thioacetamide TS; no precipitate is produced. Add, drop by drop, sodium hydroxide (~80 g/l) TS; a white, gelatinous precipitate appears that redissolves on addition of more sodium hydroxide. Add slowly ammonium chloride (100 g/l) TS; the white, gelatinous precipitate reappears.

Heavy metals. Shake 1.0 g with 5 ml of hydrochloric acid (~70 g/l) TS for 5 minutes and centrifuge. Dilute the supernatant liquid to 10 ml with water, adjust the pH, and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 40 µg/g.

Acid-insoluble impurities. To 1.0 g add 25 ml of hydrochloric acid (~70 g/l) TS and shake for 5 minutes. Filter through a tared sintered-glass filter, wash the residue with water, dry to constant mass at 105 °C, and weigh; the residue weighs not more than 20 mg.

Alkalinity. Suspend 1 g in 50 ml of water and titrate with hydrochloric acid (0.1 mol/l) VS to pH 4; not more than 10 ml is required.

ALUMINII SULFAS
ALUMINIUM SULFATE

$Al_2(SO_4)_3,nH_2O$

Relative molecular mass. 342.1 (anhydrous).

Chemical name. Aluminium sulfate (2:3); CAS Reg. No. 10043-01-3 (anhydrous). Aluminium sulfate (2:3) hydrate; CAS Reg. No. 17927-65-0 (hydrate).

Description. Colourless, lustrous crystals or crystalline masses, or a white, crystalline powder; odourless.

Solubility. Soluble in cold water; freely soluble in hot water; practically insoluble in ethanol (~750 g/l) TS.

Category. Astringent drug; used in the preparation of aluminium acetate solution.

Storage. Aluminium sulfate should be kept in a well-closed container. Aluminium sulfate contains a variable quantity of water of crystallization.

Requirements

Aluminium sulfate contains not less than **51.0%** and not more than the equivalent of **59.0%** of $Al_2(SO_4)_3$.

Identity tests

A. Dissolve 0.2 g in 2 ml of water and add 0.5 ml of hydrochloric acid (~70 g/l) TS and 0.5 ml of alkaline thioacetamide TS; no precipitate is formed. Add drop by drop sodium hydroxide (~80 g/l) TS; a white, gelatinous precipitate is formed which dissolves on further addition of sodium hydroxide. Gradually add ammonium chloride (100 g/l) TS; a white, gelatinous precipitate reappears.

B. A 0.1 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 50 µg/g.

Ammonium salts. Heat 1 g with 10 ml of sodium hydroxide (1 mol/l) VS on a water-bath for 1 minute; no odour of ammonia is perceptible.

Iron. Use 0.4g; the solution complies with the 2.2.4 Limit test for iron; not more than 100µg/g.

Alkali and alkaline-earth metals. Dissolve 1.0g in 100ml of water, heat, and add 0.1ml of methyl red/ethanol TS and sufficient ammonia (~100g/l) TS until the colour of the solution changes to yellow. Dilute to 150ml with water, heat to boiling, and filter. Evaporate 75ml of the filtrate to dryness on a water-bath and ignite to constant mass; the residue weighs not more than 2mg (0.4%).

Colour and clarity of solution. A solution of 0.50g in 10ml of water is colourless and not more opalescent than opalescence standard TS2.

pH value. pH of a 20mg/ml solution in carbon-dioxide-free water R, 2.5–4.0.

Assay. Dissolve about 0.5g, accurately weighed, in 20ml of water, and proceed as described under 2.5 Complexometric titrations for aluminium.

Each ml of disodium edetate (0.05mol/l) VS is equivalent to 8.554mg of $Al_2(SO_4)_3$.

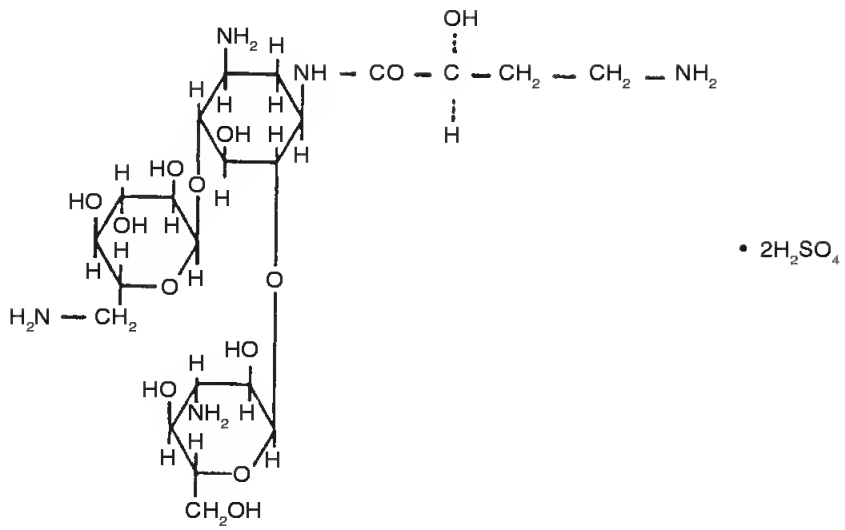
AMIKACINI SULFAS
AMIKACIN SULFATE

Amikacin sulfate (non-injectable)
Amikacin sulfate, sterile

Molecular formula. $C_{22}H_{43}N_5O_{13},2H_2SO_4$

Relative molecular mass. 781.8

Graphic formula.



Chemical name. *O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→6)]-N^3-(4-amino-L-2-hydroxybutyryl)-2-deoxy-L-streptamine sulfate (1:2) (salt); (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-N^1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine sulfate (1:2) (salt); CAS Reg. No. 39831-55-5.

Description. A white to yellowish white, crystalline powder; almost odourless.

Solubility. Very soluble in water; practically insoluble in methanol R, acetone R or ether R.

Category. Antibacterial drug.

Storage. Amikacin sulfate should be kept in a tightly closed container.

Labelling. The designation sterile Amikacin sulfate indicates that the substance complies with the additional requirements for sterile Amikacin sulfate and may be used for parenteral administration or for other sterile applications.

Requirements

Definition. Amikacin sulfate contains not less than 650 International Units per mg, with reference to the anhydrous substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS and mix, then add 2 ml of cobalt(II) nitrate (10 g/l) TS; a violet colour is produced.

B. Dissolve 0.05 g in 3 ml of water and add 4 ml of anthrone TS; a bluish violet colour is produced.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +69 to +79 °.

Sulfated ash. After ignition moisten the residue with 2 ml of nitric acid (~1000 g/l) TS and about 0.2 ml of sulfuric acid (~1760 g/l) TS; not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not more than 50 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 6.0–7.5.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus subtilis* (ATCC 6633) as the test organism, culture medium Cm1 with a final pH of 6.5–6.7, sterile phosphate buffer pH 6.0 TS1, TS2 or TS3, an appropriate concentration of amikacin (usually 5–20 IU per ml), and an incubation temperature of 32–35 °C, or (*b*) *Staphylococcus aureus* (ATCC 29737) as the test organism, the same culture medium and phosphate buffer, an appropriate concentration of amikacin (usually 10 IU per ml), and the same incubation temperature. The precision of the assay is such that the fiducial limits of error of the estimated potency (P = 0.95) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency (P = 0.95) is not less than 650 IU per mg, calculated with reference to the anhydrous substance.

Additional requirements for Amikacin sulfate for sterile use

Storage. Sterile Amikacin sulfate should be kept in a hermetically closed container.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

Additional requirements for Amikacin sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

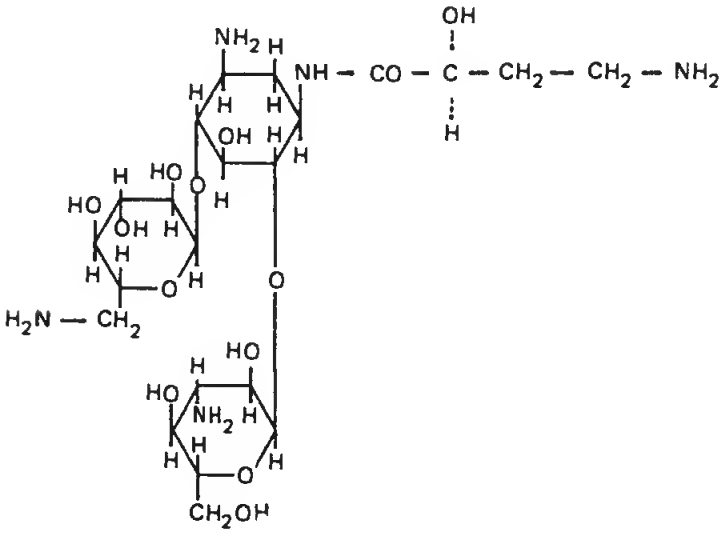
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.33 IU of endotoxin RS per mg of amikacin.

AMIKACINUM
AMIKACIN

Molecular formula. $C_{22}H_{43}N_5O_{13}$

Relative molecular mass. 585.6

Graphic formula.



Chemical name. *O*-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→6)]-N^3-(4-amino-L-2-hydroxybutyryl)-2-deoxy-L-streptamine; (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-N^1-(4-amino-2-hydroxyoxobutyl)-2-deoxy-D-streptamine; CAS Reg. No. 37517-28-5.

Description. A white, crystalline powder; almost odourless.

Solubility. Sparingly soluble in water.

Category. Antibacterial drug.

Storage. Amikacin should be kept in a tightly closed container.

Requirements

Definition. Amikacin contains not less than 900 µg of $C_{22}H_{43}N_5O_{13}$ per mg, with reference to the anhydrous substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS and mix, then add 2 ml of cobalt(II) nitrate (10 g/l) TS; a violet colour is produced.

B. Dissolve 0.05 g in 3 ml of water and add 4 ml of anthrone TS; a bluish violet colour is produced.

Specific optical rotation. Use a 20 mg/ml solution and calculate with reference to the anhydrous substance: $[\alpha]_D^{20\,°C}$ = +97 to +105 °.

Sulfated ash. After ignition moisten the residue with 2 ml of nitric acid (~1000 g/l) TS and about 0.2 ml of sulfuric acid (~1760 g/l) TS; not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not more than 85 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 9.5–11.5.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus subtilis* (ATCC 6633) as the test organism, culture medium Cm1 with a final pH of 6.5–6.7, sterile phosphate buffer pH 6.0 TS1, TS2 or TS3, an appropriate concentration of amikacin (usually 5–20 µg/ml), and an incubation temperature of 32–35 °C, or (*b*) *Staphylococcus aureus* (ATCC 29737) as the test organism, the same culture medium and phosphate buffer, an appropriate concentration of amikacin (usually 10 µg/ml), and the same incubation temperature. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 900 g per mg, calculated with reference to the anhydrous substance.

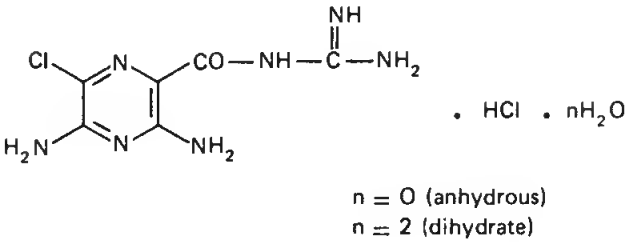
AMILORIDI HYDROCHLORIDUM
AMILORIDE HYDROCHLORIDE

Amiloride hydrochloride, anhydrous
Amiloride hydrochloride dihydrate

Molecular formula. $C_6H_8ClN_7O,HCl$ (anhydrous); $C_6H_8ClN_7O,HCl,2H_2O$ (dihydrate).

Relative molecular mass. 266.1 (anhydrous); 302.1 (dihydrate).

Graphic formula.



Chemical name. *N*-Amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride; 3,5-diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamide monohydrochloride; 3,5-diamino-6-chloro-*N*-(diaminomethylene) pyrazinecarboxamide monohydrochloride; CAS Reg. No. 2016-88-8 (anhydrous). *N*-Amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride dihydrate; 3,5-diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamide monohydrochloride dihydrate; 3,5-diamino-6-chloro-*N*-(diaminomethylene)-pyrazinecarboxamide monohydrochloride dihydrate; CAS Reg. No. 17440-83-4 (dihydrate).

Description. A pale yellow to greenish yellow powder; odourless or almost odourless.

Solubility. Slightly soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Diuretic.

Storage. Amiloride hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Amiloride hydrochloride contains not less than 98.0% and not more than 101.0% of $C_6H_8ClN_7O,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from amiloride hydrochloride RS or with the *reference spectrum* of amiloride hydrochloride.

B. The absorption spectrum of a 5.0 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 380 nm, exhibits maxima at about 285 nm and 361 nm; the absorbances of a 1-cm layer at the maximum wavelength of 285 nm and 361 nm are about 0.28 and 0.31, respectively.

C. A 5 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; for the dihydrate the water content is not less than 110 mg/g and not more than 130 mg/g.

Free acid. Dissolve 1.0 g in a mixture of 50 ml of methanol R and 50 ml of water, and titrate with sodium hydroxide (0.1 mol/l) VS determining the endpoint potentiometrically; not more than 0.3 ml is required.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 15 volumes of tetrahydrofuran R and 2 volumes of ammonia (~50 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of 4 volumes of methanol R and 1 volume of chloroform R containing (A) 0.40 mg of the test substance per ml, (B) 4.0 µg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.45 g, accurately weighed, in a mixture of 100 ml of glacial acetic acid R1, 15 ml of dioxan R, and 10 ml of mercuric acetate/acetic

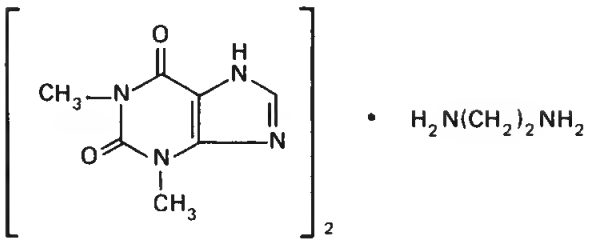
acid TS, and titrate with perchloric acid (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.61 mg of $C_6H_8ClN_7O$,HCl.

AMINOPHYLLINUM
AMINOPHYLLINE

Molecular formula. $(C_7H_8N_4O_2)_2,C_2H_8N_2$ (anhydrous) or $C_{16}H_{24}N_{10}O_4$

Relative molecular mass. 420.4 (anhydrous)

Graphic formula.



Chemical name. Theophylline compound with ethylenediamine (2:1); 3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione compound with 1,2-ethanediamine (2:1); CAS Reg. No. 317-34-0 (anhydrous).

Description. White or slightly yellowish granules or powder; odour, slightly ammoniacal.

Solubility. Freely soluble in water (the solution may become cloudy in the presence of carbon dioxide); slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antispasmodic; diuretic; coronary vasodilator.

Storage. Aminophylline should be kept in a tightly closed container, protected from light.

Additional information. Aminophylline contains a variable quantity of water of hydration. Upon exposure to air Aminophylline gradually loses ethylenediamine and absorbs carbon dioxide with the liberation of free theophylline. Even in the absence of light, Aminophylline is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Aminophylline contains not less than 78.0% and not more than 86.0% of theophylline ($C_7H_8N_4O_2$), and not less than 12.8% and not more than 15.0% of ethylenediamine ($C_2H_8N_2$), both calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 1 g in 10 ml of water and add, drop by drop, while shaking 2 ml of hydrochloric acid (~70 g/l) TS. Collect the precipitate on a filter, wash it with water and dry at 105 °C; melting temperature, about 272 °C (theophylline). Keep the precipitate for test B.

B. To 10 mg of the precipitate obtained from test A, contained in a porcelain dish, add 1 ml of hydrochloric acid (~250 g/l) TS and 0.5 ml of hydrogen peroxide (~60 g/l) TS, and evaporate to dryness on a water-bath. Add 1 drop of ammonia (~100 g/l) TS; the residue acquires a purple colour which is destroyed by the addition of a few drops of sodium hydroxide (~80 g/l) TS.

C. Dissolve 0.05 g in 1 ml of water and add 2 drops of copper(II) sulfate (80 g/l) TS; a deep violet colour is produced.

D. Warm 0.05 g with 2 ml of sodium hydroxide (~80 g/l) TS and 2 drops of chloroform R; an isocyanide, perceptible by its characteristic odour (proceed with caution), is produced.

Clarity of solution. A solution of 1.0 g in 10 ml of boiling water is clear or is not more than slightly opalescent.

Sulfated ash. Not more than 1.5 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.15 g of the substance and 25 ml of pyridine R as the solvent; the water content is not more than 80 mg/g.

Alkalinity. Add 1 drop of thymol blue/ethanol TS to a 10 mg/ml solution prepared in carbon-dioxide-free water R; a green or blue colour is produced.

Assay

For theophylline. Place about 0.25 g, accurately weighed, in a 250-ml conical flask, add 50 ml of water and 8 ml of ammonia (~100 g/l) TS and gently warm the mixture on a water-bath until complete solution is effected. Add 20.0 ml of silver nitrate (0.1 mol/l) VS, mix, heat to boiling and boil for 15 minutes. Cool to between 5 °C and 10 °C for 20 minutes, then filter through a filtering crucible under reduced pressure and wash the precipitate 3 times with 10-ml

portions of water. Acidify the combined filtrate and washings with nitric acid (~1000 g/l) TS, and add an excess of 3 ml of the acid. Cool, add 2 ml of ferric ammonium sulfate (45 g/l) TS, and titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

For ethylenediamine. Dissolve about 0.5 g, accurately weighed, in 30 ml of water and titrate with hydrochloric acid (0.1 mol/l) VS, using bromocresol green/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 3.005 mg of $C_2H_8N_2$.

Additional requirements for Aminophylline for parenteral use

Complies with the monograph for "Parenteral preparations".

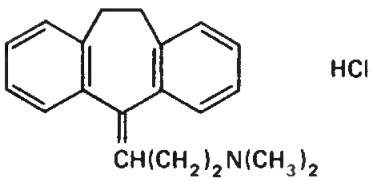
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.0 IU of endotoxin RS per mg.

AMITRIPTYLINI HYDROCHLORIDUM
AMITRIPTYLINE HYDROCHLORIDE

Molecular formula. $C_{20}H_{23}N,HCl$

Relative molecular mass. 313.9

Graphic formula.



Chemical name. 10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[*a*,*d*]cycloheptene-$\Delta^{5,\gamma}$-propylamine hydrochloride; 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine hydrochloride; CAS Reg. No. 549-18-8.

Description. Colourless crystals or a white or almost white powder; odourless or almost odourless.

Solubility. Soluble in 1 part of water and in 1.5 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antidepressant.

Storage. Amitriptyline hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Amitriptyline hydrochloride has a bitter and burning taste that is followed by a sensation of numbness. Even in the absence of light, Amitriptyline hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Amitriptyline hydrochloride contains not less than 99.0% and not more than 101.5% of $C_{20}H_{23}N,HCl$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from amitriptyline hydrochloride RS or with the *reference spectrum* of amitriptyline hydrochloride.

B. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

C. Melting temperature, about 197 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5–6.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 85 volumes of cyclohexane R, 15 volumes of ethyl acetate R and 3 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

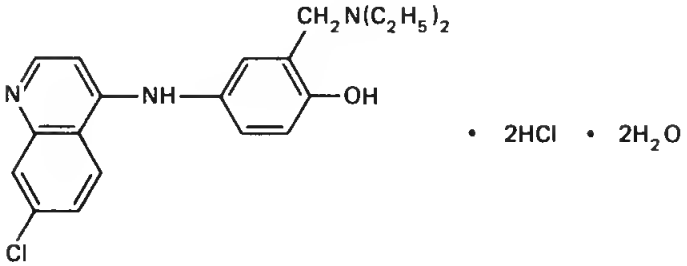
Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of dioxan R and 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 31.39 mg of $C_{20}H_{23}N,HCl$.

AMODIAQUINI HYDROCHLORIDUM
AMODIAQUINE HYDROCHLORIDE

Molecular formula. $C_{20}H_{22}ClN_3O,2HCl,2H_2O$

Relative molecular mass. 464.8

Graphic formula.



Chemical name. 4-[(7-Chloro-4-quinolyl)amino]-α-(diethylamino)-*o*-cresol dihydrochloride dihydrate; 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)-methyl]phenol dihydrochloride dihydrate; CAS Reg. No. 6398-98-7.

Description. A yellow, crystalline powder; odourless.

Solubility. Soluble in about 22 parts of water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antimalarial.

Storage. Amodiaquine hydrochloride should be kept in a tightly closed container.

Requirements

Definition. Amodiaquine hydrochloride contains not less than 98.0% and not more than 101.5% of $C_{20}H_{22}ClN_3O,2HCl$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or all 3 tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of amodiaquine hydrochloride.

B. To 1 ml of a 20 mg/ml solution add 0.5 ml of cobaltous thiocyanate TS; a green precipitate is produced.

C. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

D. Melting temperature, determined without previous drying, about 158 °C with decomposition.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.15 g of the substance; the water content is not less than 70 mg/g and not more than 90 mg/g.

pH value. pH of a 20 mg/ml solution, 4.0–4.8.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography. Prepare a solution of chloroform saturated with ammonia by shaking chloroform R with ammonia (~260 g/l) TS and separate the chloroform-layer. Use silica gel R2 as the coating substance and a mixture of 9 volumes of chloroform saturated with ammonia, and 1 volume of dehydrated ethanol R as the mobile phase. For the preparation of the test solutions transfer 0.20 g of the substance being examined to a glass-stoppered test-tube, add 10 ml of chloroform saturated with ammonia, shake vigorously for 2 minutes, allow the solids to settle, and decant the solution to a second tube; this constitutes solution A. Dilute 1.0 ml of solution A with sufficient chloroform saturated with ammonia to 200 ml; this constitutes solution B. Apply separately to the plate 10 µl of each of solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

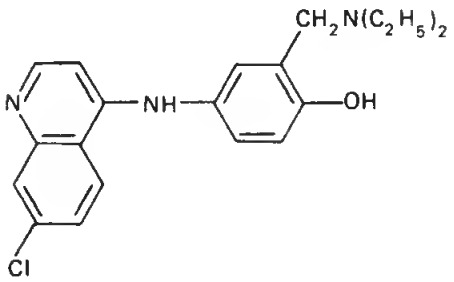
Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of water, make the solution alkaline with ammonia (~100 g/l) TS, and allow to stand for 30 minutes. Filter, wash the residue with water until the washings are free from chlorides, and dry to constant weight at 105 °C. Each g of residue is equivalent to 1.205 g of $C_{20}H_{22}ClN_3O,2HCl$.

AMODIAQUINUM
AMODIAQUINE

Molecular formula. $C_{20}H_{22}ClN_3O$

Relative molecular mass. 355.9

Graphic formula.



Chemical name. 4-[(7-Chloro-4-quinolyl)amino]-α-(diethylamino)-*o*-cresol; 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)methyl]phenol; CAS Reg. No. 86-42-0.

Description. A yellow, crystalline powder; odourless.

Solubility. Practically insoluble in water.

Category. Antimalarial drug.

Storage. Amodiaquine should be kept in a tightly closed container.

Requirements

Definition. Amodiaquine contains not less than 97.0% and not more than 103.0% of $C_{20}H_{22}ClN_3O$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from the free base of amodiaquine hydrochloride RS or with the *reference spectrum* of amodiaquine.

B. Dissolve 20 mg in 1.0 ml of water and add 0.5 ml of ammonium thiocyanate/cobalt(II) nitrate TS; a green precipitate is produced.

C. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

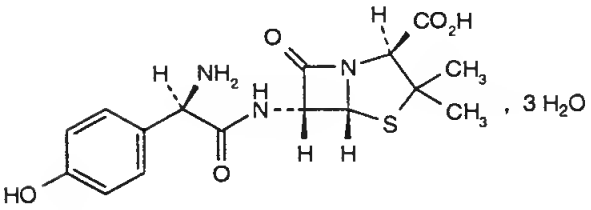
Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.8 g of the substance; the water content is not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography. Prepare a solution of chloroform saturated with ammonia by shaking chloroform R with ammonia (~260 g/l) TS and separate the chloroform layer. Use silica gel R2 as the coating substance and a mixture of 9 volumes of chloroform, saturated with ammonia, and 1 volume of dehydrated ethanol R as the mobile phase. For the preparation of the test solution dissolve 0.15 g of the substance being examined in 10 ml of chloroform saturated with ammonia (solution A). For the preparation of the reference solutions transfer 40 mg of amodiaquine hydrochloride RS to a glass-stoppered test-tube, add 2.0 ml of chloroform saturated with ammonia, and shake vigorously for 2 minutes. Allow the solids to settle, and decant the solution to a second test-tube (solution B). Dilute 1.0 ml of solution B to 200 ml with chloroform saturated with ammonia (solution C). Apply separately to the plate 10 µl of each of solutions A, B and C. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in sufficient hydrochloric acid (0.1 mol/l) VS to produce 200 ml; dilute 10.0 ml of this solution to 1000 ml with the same medium. Separately prepare a reference solution containing 15 µg of amodiaquine hydrochloride RS per ml of hydrochloric acid (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer of both solutions at the maximum at about 342 nm against a solvent cell containing hydrochloric acid (0.1 mol/l) VS. Calculate the quantity, in mg, of $C_{20}H_{22}ClN_3O$ in the substance being examined using the formula $(355.9/428.8)\ (20C)\ (A_u/A_s)$, in which 355.9 and 428.8 are the relative molecular masses of amodiaquine and anhydrous amodiaquine hydrochloride, respectively, C is the concentration, in µg per ml, calculated with reference to the anhydrous substance of amodiaquine hydrochloride RS in the reference solution, and A_u and A_s are the absorbances of the solution of the substance being examined and the reference solution, respectively.

AMOXICILLINUM TRIHYDRICUM
AMOXICILLIN TRIHYDRATE



$C_{16}H_{19}N_3O_5S,3H_2O$

Relative molecular mass. 419.5

Chemical name. (–)-6-[2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid trihydrate; (2*S*,5*R*,6*R*)-6-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; 6-[[amino (4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid trihydrate; CAS Reg. No. 61336-70-7.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/l) TS, ether R, and fatty oils; soluble in dilute acids and dilute solutions of alkali hydroxides.

Category. Antibacterial drug.

Storage. Amoxicillin trihydrate should be kept in a tightly closed container.

Requirements

Amoxicillin trihydrate contains not less than **95.0%** and not more than the equivalent of **102.0%** of $C_{16}H_{19}N_3O_5S$, calculated with reference to the anhydrous substance.

Identity tests

- *Either test A alone or tests B and C may be applied.*

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from amoxicillin trihydrate RS or with the *reference spectrum* of amoxicillin trihydrate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silanized silica gel R3 as the coating substance and a mixture of 9 volumes of a solution containing 15.4 g of ammonium acetate R in 100 ml, the pH of which has been adjusted to 5.0 with glacial acetic acid R, and 1 volume of acetone R as the mobile phase. Apply separately to the plate 1 µl of each of 3 solutions in sodium hydrogen carbonate (40 g/l) TS containing (A) 2.5 mg of Amoxicillin trihydrate per ml, (B) 2.5 mg of amoxicillin trihydrate RS per ml, and (C) a mixture of 2.5 mg of amoxicillin trihydrate RS and 2.5 mg of ampicillin trihydrate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Expose the plate to iodine vapours until the spots appear and examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows two clearly separated spots.

C. Place about 2 mg in a test-tube (150 mm × 15 mm), moisten with 1 drop of water, and add about 2 ml of sulfuric acid (~1760 g/l) TS. Mix the contents of the tube by swirling; the solution remains practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

Specific optical rotation. Use a 2.0 mg/ml solution in carbon-dioxide-free water R and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +290° to +315°

Solution in hydrochloric acid and ammonia. Prepare a solution of 1.0 g in 10 ml of hydrochloric acid (0.5 mol/l) VS. Prepare a second solution of 1.0 g in 10 ml of ammonia (~100 g/l) TS. Examine both solutions immediately.

Neither of these solutions are more opalescent than opalescence standard TS3.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.1 g of Amoxicillin trihydrate; the water content is not less than 0.115 g/g and not more than 0.145 g/g.

pH value. pH of a 2 mg/ml solution in carbon-dioxide-free water R, 3.5–5.5.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). Prepare the following pH 5 buffer solution to be used in the mobile phases: to 250 ml of potassium dihydrogen phosphate (27.2 g/l) TS add sodium hydroxide (~80 g/l) TS until a pH of 5.0 is reached, and dilute the solution with sufficient water to produce 1000 ml. As mobile phase A use a mixture of 99 volumes of buffer solution pH 5.0 and 1 volume of acetonitrile R. As mobile phase B use a mixture of 8 volumes of buffer solution pH 5.0 and 2 volumes of acetonitrile R.

Prepare the following solutions in mobile phase A: solution (A) 1.5 mg of Amoxicillin trihydrate per ml; solution (B) 0.015 mg of amoxicillin trihydrate RS per ml; and solution (C) 0.15 µg of amoxicillin trihydrate RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Using a 50-µl loop injector, inject solution B. Start the elution isocratically with the mobile phase mixture used for the equilibration. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a ratio of mobile phase A: B of 0: 100 over a period of 25 minutes. Adjust the sensitivity of the system so that the height of the principal peak is at least 50% of the full scale of the recorder. Continue the chromatography with mobile phase B for 15 minutes, then equilibrate the column for 15 minutes with the mobile phase originally used for the equilibration. The mass distribution ratio for the first peak (amoxicillin) is 1.3–2.5. Inject mobile phase A using the 50-µl loop injector and use the same elution gradient to obtain a blank. Inject solution C using the 50-µl loop injector. Adjust the system to obtain a peak with a signal-tonoise ratio of at least 3.

Using the 50-µl loop injector, inject solution A. Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak and any peak obtained in the blank chromatogram, is not greater than that of the principal peak obtained with solution B (1%).

Assay. Dissolve about 0.06 g, accurately weighed, in sufficient water to produce 500 ml. Simultaneously, prepare a reference solution containing 0.06 g of amoxicillin trihydrate RS. Transfer 10.0 ml of one solution to a 100-ml volumetric flask and 10.0 ml of the other solution to a second 100-ml volumetric flask. To each add 10 ml of buffer borate, pH 9.0, TS and 1 ml of acetic anhydride/ dioxan TS, mix, allow to stand for 5 minutes at room temperature, and dilute to volume with water. Transfer two 2.0 ml aliquots of each solution to separate stoppered test-tubes. To one tube containing the test solution, and to

the other, containing the reference solution, add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tubes, and place them in a water-bath at 60 °C for exactly 25 minutes. Cool the tubes rapidly to 20 °C (*solution A*). To the remaining tubes add 10 ml of water and mix (*solution B*). Without delay, measure the absorbances of a 1-cm layer at the maximum at about 325 nm of both *solutions A*, using as a blank a mixture of 2.0 ml of water and 10 ml of imidazole/mercuric chloride TS placed in the solvent cell. For *solutions B* use water as a blank placed in the solvent cell.

From the difference between the absorbances of *solutions A* and *solutions B*, calculate the percentage content of $C_{16}H_{19}N_3O_5S$ by comparison with amoxicillin trihydrate RS, with reference to the anhydrous substance.

AMPHOTERICINUM B

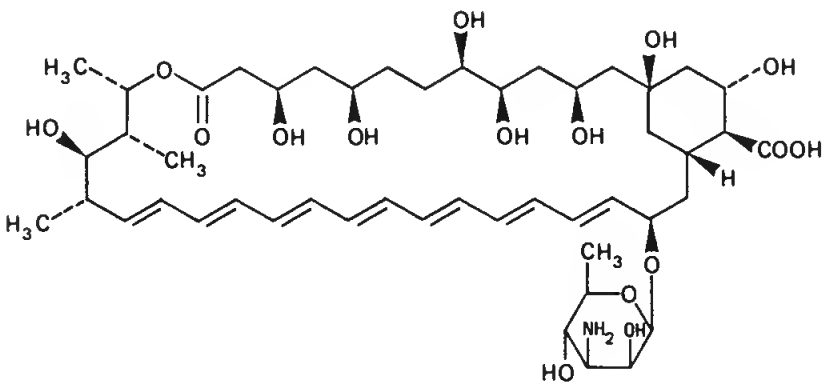
AMPHOTERICIN B

Amphotericin B for parenteral use

Molecular formula. $C_{47}H_{73}NO_{17}$

Relative molecular mass. 924.1

Graphic formula.



Chemical name. (1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-Amino-3,6-dideoxy-β-D-mannopyranosyl)-oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid; [1*R*-(1*R**,3*S**,5*R**,6*R**,9*R**,11*R**,15*S**,16*R**,17*R**,18*S**,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R**,35*S**,36*R**,37*S**)]-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-

dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid; (3*R*,5*R*,8*R*,9*R*,11*S*,13*R*,15*S*,16*R*,17*S*,19*R*,34*S*,35*R*,36*R*,37*S*)-19-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-16-carboxy-3,5,8,9,11,13,15,35-octahydroxy-34,36-dimethyl-13,17-epoxyoctatriaconta-20,22,24,26,28,30,32-heptaen-37-olide; CAS Reg. No. 1397-89-3.

Description. A yellow to orange powder; odourless or almost odourless.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, toluene R and ether R; soluble in 200 parts of dimethylformamide R and in 20 parts of dimethyl sulfoxide R, slightly soluble in methanol R.

Category. Antifungal drug.

Storage. Amphotericin B should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Labelling. The designation Amphotericin B for parenteral use indicates that the substance complies with the altered and additional requirements for Amphotericin B and may be used for parenteral administration.

Additional information. Even in the absence of light, Amphotericin B is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. In diluted solutions it is sensitive to light and is inactivated at low pH values.

Requirements

Definition. Amphotericin B contains not less than 750 µg per mg, calculated with reference to the dried substance.

Identity tests

A. Dissolve 25 mg in 5 ml of dimethyl sulfoxide R, add sufficient methanol R to produce 50 ml, and dilute 2.0 ml to 200 ml with methanol R. The absorption spectrum of the resulting solution, when observed between 300 nm and 450 nm, exhibits 3 maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance of a 1-cm layer at 362 nm to that at 381 nm is about 0.6; the ratio of the absorbance at 381 nm to that at 405 nm is about 0.9.

B. Dissolve about 1 mg in 2.0 ml of dimethyl sulfoxide R and introduce 5 ml of phosphoric acid (~1440 g/l) TS to form a lower layer; a blue ring is immediately formed at the interface of the two liquids. Mix the two liquids; a strong blue colour is produced. Add 15 ml of water and mix; the colour of the solution changes to pale yellow.

Sulfated ash. Not more than 30 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 50 mg/g.

Content of tetraenes. Dissolve 0.05 g, accurately weighed, in 5 ml of dimethyl sulfoxide R, and add sufficient methanol R to produce 50 ml; dilute 4 ml to 50 ml with methanol R (solution A). For the reference solutions dissolve similarly 0.05 g, accurately weighed, of amphotericin B RS instead of the substance being examined (solution B). Further prepare a solution of 25 mg of nystatin RS, accurately weighed, in 25 ml of dimethyl sulfoxide R, and add sufficient methanol R to produce 250 ml; dilute 4 ml to 50 ml with methanol R (solution C). Measure the absorbances of a 1-cm layer of solutions A, B, and C at the maxima at about 282 nm and 304 nm, using as a blank a solution of 5 ml of dimethyl sulfoxide R diluted to 50 ml with methanol R, 4 ml of which are diluted once again to 50 ml with methanol R.

Calculate the $A_{1\,cm}^{1\%}$ of solutions A, B, and C at both wavelengths and then apply the following formula: $F + 1000(B_1A_2 - B_2A_1)/(C_2B_1 - C_1B_2)$, where A_1 and A_2 are the $A_{1\,cm}^{1\%}$ of the substance being examined at 282 nm and 304 nm, respectively, B_1 and B_2 are the $A_{1\,cm}^{1\%}$ of amphotericin B RS at 282 nm and 304 nm, respectively, C_1 and C_2 are the $A_{1\,cm}^{1\%}$ of nystatin RS at 282 nm and 304 nm, respectively, and F is the declared content of tetraenes in amphotericin B RS; the content of tetraenes in the substance examined is not more than 150 mg/g.

Assay. Triturate 0.060 g with dimethylformamide R and add, with shaking, sufficient dimethylformamide R to produce 100 ml. Dilute 10 ml to 100 ml with dimethylformamide R and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Saccharomyces cerevisiae* (NCTC 10716, or ATCC 9763) as the test organism, culture medium Cm3 with a final pH of 6.1, sterile phosphate buffer pH 10.5, TS1, an appropriate concentration of amphotericin B (usually between 0.5 and 10.0 µg/ml), and an incubation temperature of 29–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 750 µg per mg, calculated with reference to the dried substance.

Additional requirements for Amphotericin B for parenteral use

Complies with the monograph for "Parenteral preparations".

Sulfated ash. Not more than 5.0 mg/g.

Content of tetraenes. Not more than 100 mg/g.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.9 IU of endotoxin RS per mg.

AMPICILLINUM

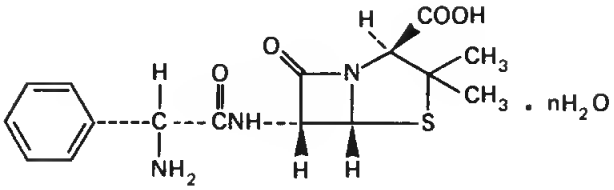
AMPICILLIN

Ampicillin anhydrous
Ampicillin trihydrate

Molecular formula. $C_{16}H_{19}N_3O_4S$ (anhydrous); $C_{16}H_{19}N_3O_4S,3H_2O$ (trihydrate).

Relative molecular mass. 349.4 (anhydrous); 403.5 (trihydrate).

Graphic formula.



n = 0 (anhydrous)
n = 3 (trihydrate)

Chemical name. (2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; [2*S*-[2α,5α,6β(*S**)]]-6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid; CAS Reg. No. 69-53-4 (anhydrous).

(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; [2*S*-[2α,5α,6β(*S**)]]-6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; CAS Reg. No. 7177-48-2 (trihydrate).

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Slightly soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Antibiotic.

Storage. Ampicillin should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container of Ampicillin should state whether the substance is in the anhydrous form or is the trihydrate.

Additional information. Even in the absence of light, Ampicillin is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ampicillin contains not less than 95.0% and not more than 102.0% of $C_{16}H_{19}N_3O_4S$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For the anhydrous form the infrared absorption spectrum is concordant with the spectrum obtained from ampicillin RS or with the *reference spectrum* of ampicillin.

   For the trihydrate the infrared absorption spectrum is concordant with the spectrum obtained from ampicillin trihydrate RS or with the *reference spectrum* of ampicillin trihydrate.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is colourless to slightly pink. Immerse the test-tube for 1 minute in a water-bath; an orange-yellow colour is produced.

Specific optical rotation. Use a 2.5 mg/ml solution and calculate with reference to the anhydrous substance: $[\alpha]_D^{20\,°C}$ = +280 to +305 °.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A.

For the anhydrous form use about 0.8 g of the substance; the water content is not more than 15 mg/g.

For the trihydrate use about 0.1 g of the substance; the water content is not less than 120 mg/g and not more than 150 mg/g.

pH value. pH of a 2.5 mg/ml solution, 3.5–6.0.

Assay. Dissolve about 0.12 g, accurately weighed, in sufficient water to produce 500 ml. Transfer 10.0 ml of this solution to a 100-ml volumetric flask, add 10 ml of buffer borate, pH 9.0, TS, 1 ml of acetic anhydride/dioxan TS, allow to stand for 5 minutes at room temperature and dilute to volume with water.

Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay, measure the absorbance of a 1-cm layer at the maximum at about 325 nm, against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{19}N_3O_4S$ in the substance being tested by comparison with ampicillin RS similarly and concurrently examined. In an adequate calibrated spectrophotometer the absorbance of the reference solution should be 0.29 ± 0.02.

AMPICILLINUM NATRICUM

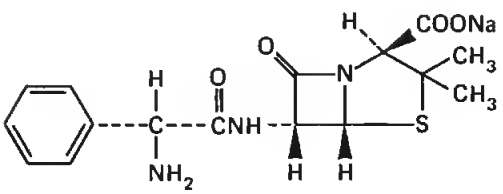
AMPICILLIN SODIUM

Ampicillin sodium (non-injectable)
Ampicillin sodium, sterile

Molecular formula. $C_{16}H_{18}N_3NaO_4S$

Relative molecular mass. 371.4

Graphic formula.



Chemical name. Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; sodium [2*S*-[2α,5α,6β(*S**)]]-6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; CAS Reg. No. 69-52-3.

Description. A white or almost white powder; odourless.

Solubility. Soluble in about 2 parts of water; practically insoluble in ether R.

Category. Antibiotic.

Storage. Ampicillin sodium should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Ampicillin sodium indicates that the substance complies with the requirements for sterile Ampicillin sodium and may be used for parenteral administration or for other sterile applications.

Additional information. Ampicillin sodium is a crystalline or amorphous powder; it is very hygroscopic and is deliquescent in air with a relative humidity of 60% or more. Even in the absence of light, Ampicillin sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ampicillin sodium contains not less than 85.0% and not more than 96.0% of $C_{16}H_{19}N_3O_4S$, calculated with reference to the anhydrous substance. Furthermore, the sum of the percentage of $C_{16}H_{19}N_3O_4S$ determined in the assay and the percentage of iodine-absorbing compounds, both calculated with reference to the anhydrous substance, is not less than 90.0%.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ampicillin sodium RS or with the *reference spectrum* of ampicillin sodium.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; a dark yellow colour is produced.

C. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, ignite a small quantity and dissolve the residue in acetic acid (~60 g/l) TS.

Specific optical rotation. Use a 5.0 mg/ml solution in acetate standard buffer TS and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +260 to +290 °.

Clarity of solution. A freshly prepared solution of 1.0 g in 10 ml of water is clear. A solution of 1.0 g in 10 ml of hydrochloric acid (1 mol/l) VS is also clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 20 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 8.0–10.0.

Iodine-absorbing compounds. Dissolve 0.25 g in sufficient water to produce 100 ml. To 10 ml of this solution add 0.5 ml of hydrochloric acid (1 mol/l) VS and 10 ml of iodine (0.01 mol/l) VS and titrate with sodium thiosulfate (0.02 mol/l) VS, using starch TS as indicator, added towards the end of the titration. Repeat the procedure without the substance being examined; the difference between the titrations represents the amount of iodine-absorbing compounds. Calculate as a percentage the amount of these compounds in the examined substance, taking into account that each ml of sodium thiosulfate (0.02 mol/l) VS is equivalent to 0.7392 mg of iodine-absorbing compounds expressed as $C_{16}H_{19}N_3O_4S$.

Assay. Dissolve about 0.12 g, accurately weighed, in sufficient water to produce 500 ml. Transfer 10.0 ml of this solution to a 100-ml volumetric flask, add 10 ml of buffer borate, pH 9.0, TS, 1 ml of acetic anhydride/dioxan TS, allow to stand for 5 minutes at room temperature and dilute to volume with water.

Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate as a percentage the amount of $C_{16}H_{19}N_3O_4S$ in the substance being tested by comparison with ampicillin RS similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.29 ± 0.02.

Additional requirements for Ampicillin sodium for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.15 IU of endotoxin RS per mg of ampicillin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

AMYLA
STARCHES

Chemical name. Starch; CAS Reg. No. 9005-25-8.

Description. A white to slightly yellowish, fine powder or ovoid granules whose size and shape are characteristic for each botanical variety; odourless.

Solubility. Practically insoluble in cold water and ethanol (~750 g/l) TS.

Category. Tablet and capsule binder; diluent; disintegrant.

Storage. Starches should be kept in tightly closed containers.

Labelling. The designation on the container of starches should state the botanical source.

Additional information. Types of starches should not be interchanged since the properties are characteristic for each one obtained from different botanical sources and thus their performance may not be identical. Attention should be paid to the microbiological purity since starches are of natural origin.

Requirements

Definition. Starches consist of polysaccharide granules obtained from the grains of corn (*Zea mays* L.), of rice (*Oryza sativa* L.), of wheat (*Triticum aestivum* L.), or from the tubers of the potato (*Solanum tuberosum* L.).

Identity tests

A. To 1 g add 50 ml of water, heat to boiling for 1 minute, and cool; a thin cloudy mucilage is obtained from all starches other than potato starch, which gives a thicker and more translucent mucilage. (Keep the mucilages for test B.)

B. To 1 ml of the mucilage obtained in test A add 0.05 ml of iodine (0.005 mol/l) VS and mix; a dark blue colour is obtained which disappears on heating and reappears on cooling.

Microscopic examination
Corn starch – Angular polyhedral or rounded granules, up to 35 µm in diameter; central hilum consisting of a distinct cavity or several rayed central clefts.

Rice starch – Polyhedral granules, size 2–10 µm, either isolated or aggregated in ovoid masses; central hilum poorly visible.

Wheat starch – Two distinct types of granule, either simple lenticular, 20–50 µm in diameter, or small spherical, 5–10 µm in diameter; hilum and striations poorly visible.

Potato starch – Simple irregular, ovoid, or spherical granules, up to 100 µm in size; hilum near the narrower end; striations well marked and concentric.

Iron. Shake 1.5 g with 15 ml of hydrochloric acid (~70 g/l) TS and filter. The filtrate complies with the 2.2.4 Limit test for iron; not more than 10 µg/g.

Sulfated ash
Corn starch – not more than 6 mg/g.

Rice starch – not more than 10 mg/g.

Wheat starch – not more than 6 mg/g.

Potato starch – not more than 6 mg/g.

Loss on drying. Dry to constant mass at 100 °C:

Corn starch – not more than 150 mg/g.

Rice starch – not more than 150 mg/g.

Wheat starch – not more than 150 mg/g.

Potato starch – not more than 200 mg/g.

Acidity. To 10 g add 100 ml of ethanol (~600 g/l) TS previously neutralized using phenolphthalein/ethanol TS, shake for 1 hour, filter, and titrate 50 ml of the filtrate with sodium hydroxide (0.1 mol/l) VS; not more than 2.0 ml is required to change the colour of the solution.

Foreign matter. Using a microscope, not more than traces of cell debris are present, and there are no granules of any origin other than that stated on the label.

Oxidizing matter. Shake 5 g with a mixture of 10 ml of water and 1.2 ml of acetic acid (~300 g/l) TS until a suspension of homogenous appearance is obtained. Add 0.5 ml of a freshly prepared saturated solution of potassium iodide R; no blue, brown, or red colour is observed.

Sulfur dioxide. Mix 20 g with 200 ml of water until a suspension of homogenous appearance is obtained. Filter. To 100 ml of the clear filtrate add 3 ml of starch TS and titrate with iodine (0.005 mol/l) VS until a permanent blue colour is obtained; not more than 2.7 ml is required (0.08 mg/g).

AQUA PRO INJECTIONE
WATER FOR INJECTIONS

Description. A clear and colourless liquid; odourless.

Category. Solvent.

Labelling. The designation on the container should indicate that water for injections is non-sterile.

Storage. Water for injections should be kept in a well-closed container.

Additional information. *CAUTION*: Water for injections is not sterile. It is not a final dosage form, but an intermediate product in the manufacture of parenteral preparations, either for bulk injectable solutions or for "Sterile water for injections".

Requirements

Definition. Water for injections is pyrogen-free. It contains no added substance.

Manufacture. Water for injections is obtained from potable or Purified water by distillation in an apparatus of which the parts in contact with the liquid are of neutral glass, quartz, or suitable metal and fitted with an effective device to prevent entrainment of droplets. The first portion of the distillate obtained when the apparatus begins to function is discarded. The distillate is collected and stored in conditions designed to prevent growth of microorganisms and to avoid any other contamination.

Heavy metals. Use 40 ml, adjust the pH with acetic acid (~60 g/l) PbTS, and proceed as described under 2.2.3 Limit test for heavy metals, procedure 1; determine the heavy metals content according to Method A, allowing to stand for 10 minutes; the colour is not darker than that of 40 ml of the same untreated Water for injections, the pH of which has been similarly adjusted.

Ammonia. Transfer 50 ml to a comparison tube, add 2 ml of alkaline potassiomercuric iodide TS, and observe down the vertical axis of the tube in diffused light against a white background; the colour produced is not more intense than that of 50 ml of ammonia-free water R with the addition of 2 ml of dilute ammonium chloride TS.

Calcium and magnesium. To 100 ml add 2 ml of ammonium chloride buffer, pH 10.0, TS, 50 mg of mordant black 11 R, and 0.5 ml of disodium edetate (0.01 mol/l) VS; a pure blue colour is produced.

Carbon dioxide. To 25 ml add 25 ml of calcium hydroxide TS; it remains clear.

Chlorides. To 10 ml add 1 ml of silver nitrate (40 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Nitrates. Carefully superimpose 5 ml on 5 ml of diphenylamine/sulfuric acid TS, ensuring that the liquids do not mix; no blue colour appears at the interface of the two liquids.

Sulfates. To 10 ml add 1 ml of barium chloride (50 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Oxidizable matter. To 100 ml add 10 ml of sulfuric acid (~100 g/l) TS and 0.2 ml of potassium permanganate (0.02 mol/l) VS and boil for 3 minutes; the colour is not completely destroyed.

Non-volatile residue. Boil 500 ml on a hot plate or over a flame until the volume is reduced to about 50 ml, then evaporate on a water-bath to dryness. Dry the residue for 1 hour at 105 °C; not more than 5 mg (0.01 mg/ml).

Acidity or alkalinity. To 10 ml add 2 drops of methyl red/ethanol TS; no red colour appears. To a further 10 ml portion add 5 drops of bromothymol blue/ethanol TS; no blue colour develops.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.25 IU of endotoxin RS per ml.

AQUA PURIFICATA
PURIFIED WATER

H_2O

Relative molecular mass. 18.02

Chemical name. Water; CAS Reg. No. 7732-18-5.

Description. A clear, colourless liquid; odourless.

Category. Solvent.

Storage. Purified water should be kept in a well-closed container.

Labelling. The designation on the container should indicate the method of preparation.

Additional information. *CAUTION*:

— Purified water must not be used for preparations intended for parenteral administration.

— Purified water intended for ophthalmic preparations must be sterilized immediately before use (see 3.2 Test for sterility and 5.8 Methods of sterilization).

Requirements

Definition. Purified water contains no added substance.

Manufacture. Purified water is prepared by distillation, ion-exchange treatment, reverse osmosis, or other appropriate process from suitable water.

Heavy metals. Use 40 ml, adjust the pH with acetic acid (~60 g/l) PbTS and proceed as described under 2.2.3 Limit test for heavy metals, procedure 1; determine the heavy metals content according to Method A, allowing to stand for 10 minutes; the colour is not darker than that of 40 ml of the same untreated Purified water, the pH of which has been similarly adjusted.

Ammonia. Transfer 50 ml to a comparison tube, add 2 ml of alkaline potassiomercuric iodide TS, and observe down the vertical axis of the tube in diffused light against a white background; the colour produced is not more intense than that of 50 ml of ammonia-free water R with the addition of 2 ml of dilute ammonium chloride TS.

Calcium and magnesium. To 100 ml add 2 ml of ammonium chloride buffer, pH 10.0, TS, 50 mg of mordant black 11 R, and 0.5 ml of disodium edetate (0.01 mol/l) VS; a pure blue colour is produced.

Carbon dioxide. To 25 ml add 25 ml of calcium hydroxide TS; it remains clear.

Chlorides. To 10 ml add 1 ml of silver nitrate (40 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Nitrates. Carefully superimpose 5 ml on 5 ml of diphenylamine/sulfuric acid TS, ensuring that the liquids do not mix; no blue colour appears at the interface of the two liquids.

Sulfates. To 10 ml add 1 ml of barium chloride (50 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Oxidizable matter. To 100 ml add 10 ml of sulfuric acid (~100 g/l) TS and 0.5 ml of potassium permanganate (10 g/l) TS and boil for 3 minutes; the colour is not completely destroyed.

Non-volatile residue. Evaporate 500 ml on a water-bath to dryness and dry the residue for 1 hour at 105 °C; not more than 5 mg (0.01 mg/ml).

Acidity or alkalinity. To 10 ml add 2 drops of methyl red/ethanol TS; a red colour does not appear. To a further 10 ml portion add 5 drops of bromothymol blue/ethanol TS; no blue colour develops.

Additional requirement for Purified water for sterile use

Complies with 3.2.1 Test for sterility of non-injectable preparations.

AQUA STERILISATA PRO INJECTIONE
STERILE WATER FOR INJECTIONS

Description. A clear and colourless liquid; odourless.

Category. Solvent (for extemporaneous use).

Storage. Sterile water for injections should be kept in a single dose container the size of which is not larger than 1 litre.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Sterile water for injections is Water for injections that is pyrogen-free and sterile. It contains no added substance. It is suitably packaged.

Manufacture. (see 5.8 Methods of sterilization).

Heavy metals. Use 40 ml, adjust the pH with acetic acid (~60 g/l) PbTS, and proceed as described under 2.2.3 Limit test for heavy metals, procedure 1; determine the heavy metals content according to Method A, allowing to stand for 10 minutes; the colour is not darker than that of 40 ml of the same untreated Sterile water for injections, the pH of which has been similarly adjusted.

Ammonia. Transfer 50 ml to a comparison tube, add 2 ml of alkaline potassio-mercuric iodide TS, and observe down the vertical axis of the tube in diffused light against a white background; the colour produced is not more intense than that of 50 ml of ammonia-free water R with the addition of 2 ml of dilute ammonium chloride TS.

Calcium and magnesium. To 100 ml add 2 ml of ammonium chloride buffer, pH 10.0, TS, 50 mg of mordant black 11 R, and 0.5 ml of disodium edetate (0.01 mol/l) VS; a pure blue colour is produced.

Carbon dioxide. To 25 ml add 25 ml of calcium hydroxide TS; it remains clear.

Chlorides. To 10 ml add 1 ml of silver nitrate (40 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Nitrates. Carefully superimpose 5 ml on 5 ml of diphenylamine/sulfuric acid TS, ensuring that the liquids do not mix; no blue colour appears at the interface of the two liquids.

Sulfates. To 10 ml add 1 ml of barium chloride (50 g/l) TS and allow to stand for 5 minutes; it remains clear and colourless.

Oxidizable matter. To 100 ml add 10 ml of sulfuric acid (~100 g/l) TS and 0.5 ml of potassium permanganate (10 g/l) TS and boil for 3 minutes; the colour is not completely destroyed.

Non-volatile residue. Evaporate 500 ml on a water-bath to dryness and dry the residue for 1 hour at 105 °C; not more than 5 mg (0.01 mg/ml).

Acidity or alkalinity. To 10 ml add 2 drops of methyl red/ethanol TS; no red colour appears. To a further 10 ml portion add 5 drops of bromothymol blue/ethanol TS; no blue colour develops.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.25 IU of endotoxin RS per ml.

Sterility. Complies with 3.2 Test for sterility.

ARGENTI NITRAS
SILVER NITRATE

Molecular formula. $AgNO_3$

Relative molecular mass. 169.9

Chemical name. Silver(1+) nitrate; CAS Reg. No. 7761-88-8.

Description. Colourless or white crystals or white cylindrical rods; odourless.

Solubility. Soluble in 0.5 parts of water; soluble in ethanol (~750 g/l) TS.

Category. Antiinfective agent.

Storage. Silver nitrate should be kept in a tightly closed, non-metallic container, protected from light.

Additional information. Even in the absence of light, Silver nitrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. On exposure to light and in the presence of organic matter, it becomes grey or greyish black.

Requirements

Definition. Silver nitrate contains not less than 99.0% and not more than 100.5% of $AgNO_3$.

Identity tests

A. Dissolve 20 mg in 1.0 ml of water, add ammonia (~100 g/l) TS, drop by drop, until the precipitate first formed just dissolves; add about 0.1 ml of formaldehyde TS and warm the mixture; glossy metallic silver forms on the wall of the test-tube.

B. Dissolve 20 mg in 1.0 ml of water and add a few drops of potassium iodide (~80 g/l) TS; a cream-coloured precipitate is produced which is insoluble in ammonia (~100 g/l) TS and nitric acid (~1000 g/l) TS.

C. To 2 ml of a 0.05 g/ml solution add 2 ml of ferrous sulfate (15 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of nitrates.

Clarity and colour. A solution of 0.4 g in 10 ml of water is clear and colourless.

Acidity or alkalinity. Dissolve 0.4 g in 10 ml of water; to a 2-ml portion add 0.1 ml of bromocresol green/ethanol TS; the colour of the solution is blue. To another 2-ml portion of the test solution add 0.1 ml of phenol red/ethanol TS; the colour of the solution is yellow.

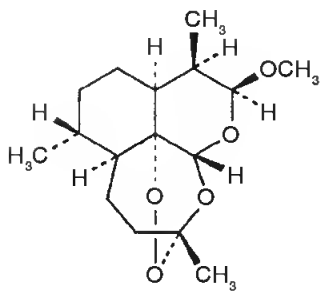
Foreign salts. Dissolve 1.2 g in 30 ml of water, add 7.5 ml of hydrochloric acid (~70 g/l) TS, shake vigorously, heat on a water-bath for 5 minutes and filter. Evaporate 20 ml of the filtrate to dryness on a water-bath and dry at 105 °C; the residue weighs not more than 2.0 mg.

Bismuth, copper, and lead. Dissolve 1.0 g in 5 ml of water, add drop by drop ammonia (~100 g/l) TS until the precipitate first formed just dissolves; the solution is clear and colourless.

Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of water, add 2 ml of nitric acid (~130 g/l) TS and 4 ml of ferric ammonium sulfate (45 g/l) TS. Titrate with ammonium thiocyanate (0.1 mol/l) VS until a reddish yellow colour is produced. Each ml of ammonium thiocyanate (0.1 mol/l) VS is equivalent to 16.99 mg of $AgNO_3$.

ARTEMETHERUM
ARTEMETHER

$C_{16}H_{26}O_5$



Relative molecular mass. 298.4

Chemical name. (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin; CAS Reg. No. 71963-77-4.

Description. White crystals or a white, crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R and acetone R; freely soluble in ethyl acetate R and dehydrated ethanol R.

Category. Antimalarial drug.

Storage. Artemether should be kept in a tightly closed container, protected from light and stored in a cool place.

Labelling. The designation Artemether for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration.

Additional information. The parenteral form is normally intended for intramuscular administration.

Requirements

Artemether contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{16}H_{26}O_5$ using Assay method A, and not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{16}H_{26}O_5$ using Assay method B, both calculated with reference to the dried substance.

Identity tests

- Either tests A and B or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To 30 mg add about 1 ml of dehydrated ethanol R and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. Dissolve 30 mg in 6.0 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Melting range. 86.0–90.0 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R;

$[\alpha]_D^{20\,°C} = +166°$ to $+173°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 5.0 mg/g.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R containing (A) 10 mg of Artemether per ml, (B) 0.05 mg of Artemether per ml, (C) 0.025 mg of Artemether per ml, (D) 0.10 mg of Artemether per ml, and (E) 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 10 mg of Artemether per ml; solution (B) 10 mg of artemether RS per ml; and for solution (C) dilute solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{16}H_{26}O_5$ with reference to the dried substance.

B. Dissolve about 0.050 g of Artemether, accurately weighed, in sufficient dehydrated ethanol R to produce 100 ml. Dilute 2 ml of this solution to 100 ml with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 °C for 5 hours. Allow to cool to room temperature.

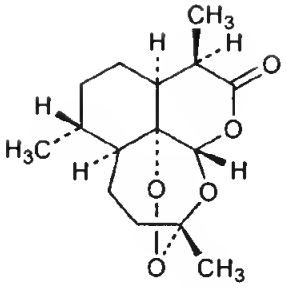
Measure the absorbance of this solution in a 1-cm layer at the maximum at about 254 nm. Calculate the percentage content of $C_{16}H_{26}O_5$ by comparison with artemether RS, similarly and concurrently examined, and with reference to the dried substance.

Additional requirement for Artemether for parenteral use

Complies with the monograph for "Parenteral preparations" and with 5.6 Test for extractable volume for parenteral preparations, and 5.7 Visual inspection of particulate matter in injectable preparations.

ARTEMISININUM
ARTEMISININ

$C_{15}H_{22}O_5$



Relative molecular mass. 282.3

Chemical name. (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4.3-*j*]-1,2-benzodioxepin-10(3*H*)-one; CAS Reg. No. 63968-64-9.

Description. Colourless needles or a white, crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R; freely soluble in acetone R and ethyl acetate R; soluble in glacial acetic acid R, methanol R and ethanol (~750 g/l) TS.

Category. Antimalarial drug.

Storage. Artemisinin should be kept in a well-closed container, protected from light and stored in a cool place.

Requirements

Artemisinin contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{22}O_5$ using Assay method A, and not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{22}O_5$ using Assay method B, both calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemisinin RS or with the *reference spectrum* of artemisinin.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

D. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of starch TS; a violet colour is immediately produced.

Melting range. 151–154 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R;

$[\alpha]_D^{20\,°C} = +75°$ to $+78°$

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 80 °C; it loses not more than 5.0 mg/g.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (µmm). The mobile phases for gradient elution consist of a mixture of acetonitrile and water, using the conditions shown in the following table:

Time (min)	Mobile phase A (% v/v of acetonitrile)	Mobile phase B (% v/v of water)	Comment
0–17	60	40	Isocratic
17–30	60 ⇒ 100	40 ⇒ 0	Linear gradient
30–35	100 ⇒ 60	0 ⇒ 40	Return to initial conditions
35–45	60	40	Isocratic – re-equilibration

Prepare the following solutions. For solution (A) use 10 mg of Artemisinin per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water,

and for solution (B) use 50 µg of Artemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare solution (C) containing 1 mg of artemisinin RS per ml and 1 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution B (0.25%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R containing (A) 10 mg of Artemisinin per ml, (B) 0.05 mg of Artemisinin per ml, (C) 0.025 mg of Artemisinin per ml, (D) 0.10 mg of Artemisinin per ml, and (E) 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/sulfuric acid TS, and heat the plate to 105 °C for 7 minutes. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel,

the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Artemisinin per ml; and solution (B) 1.0 mg of artemisinin RS per ml.

For the system suitability test prepare solution (C) containing 1 mg of artemisinin RS per ml and 1 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

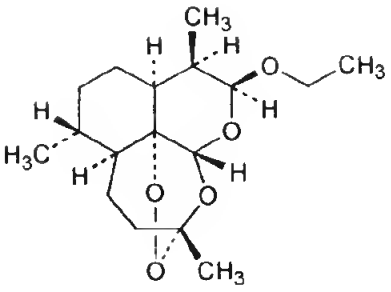
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{15}H_{22}O_5$ with reference to the dried substance.

B. Dissolve about 0.05 g of Artemisinin, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml, and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $C_{15}H_{22}O_5$ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined, and with reference to the dried substance.

ARTEMOTILUM
ARTEMOTIL

$C_{17}H_{28}O_5$



Relative molecular mass. 312.4

Chemical name. (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-ethoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin; CAS Reg. No. 75887-54-6.

Other names. Arteether, β-arteether.

Description. A white or almost white, crystalline powder.

Solubility. Practically insoluble in water; sparingly soluble in dichloromethane R, ethanol (~750 g/l) TS and methanol R; soluble in arachis oil R and sesame oil R.

Category. Antimalarial drug.

Storage. Artemotil should be kept in a well-closed container, protected from light.

Labelling. The designation Artemotil for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration.

Additional information. The parenteral form is normally intended for intramuscular administration.

Requirements

Artemotil contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{17}H_{28}O_5$ calculated with reference to the dried substance.

Identity tests

- Either tests A and B or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemotil RS or with the *reference spectrum* of artemotil.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To 30 mg add about 1 ml of dehydrated ethanol R and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. Dissolve 30 mg in 6.0 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Melting range. 81.0–84.0 °C.

Specific optical rotation. Use a 20 mg/ml solution in dehydrated ethanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +155 ° to + 157 °

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 5.0 mg/g.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than

twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R containing (A) 10 mg of Artemotil per ml, (B) 0.05 mg of Artemotil per ml, (C) 0.025 mg of Artemotil per ml, (D) 0.10 mg of Artemotil per ml, and (E) 0.10 mg of artemotil RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/ sulfuric acid TS1. Examine the chromatogram in daylight.

   Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in acetonitrile R: solution (A) 10 mg of Artemotil per ml; solution (B) 10 mg of artemotil RS per ml; and for solution (C) dilute solution A to obtain a concentration equivalent to 0.05 mg of Artemotil per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

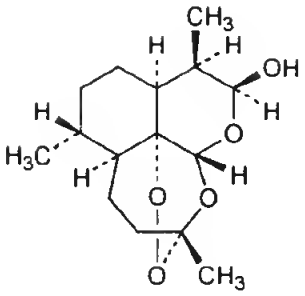
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{17}H_{28}O_5$ with reference to the dried substance.

Additional requirement for Artemotil for parenteral use

Complies with the monograph for "Parenteral preparations" and with 5.6 Test for extractable volume for parenteral preparations, and 5.7 Visual inspection of particulate matter in injectable preparations.

ARTENIMOLUM
ARTENIMOL

$C_{15}H_{24}O_5$



Relative molecular mass. 284.4

Chemical name. (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol; CAS Reg. No. 81496-81-3.

Other names. Dihydroartemisinin, b-dihydroartemisinin.

Description. Colourless needles or a white or almost white, crystalline powder.

Solubility. Practically insoluble in water; slightly soluble in acetonitrile R, ethanol (~750 g/l) TS and dichloromethane R.

Category. Antimalarial drug.

Storage. Artenimol should be kept in a well-closed container, protected from light.

Requirements

Artenimol contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{24}O_5$ using Assay method A, and not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{24}O_5$ using Assay method B, both calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with

the spectrum obtained from artenimol RS or with the *reference spectrum* of artenimol.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

D. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, and 4 drops of starch TS; a violet colour is immediately produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 10.0 mg/g.

Related substances

- Either test A or test B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (µmm). As the mobile phase for gradient elution, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water for the first 17 minutes; then run a gradient, which should reach 100% acetonitrile within 13 minutes.

Prepare the following solutions in methanol R with sonication. For solution (A) use 10 mg of Artenimol per ml in and for solution (B) use 50 µg of Artenimol per ml.

For the system suitability test prepare solution (C) by dissolving 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in methanol R with sonication.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak (twin-peak) responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the twin peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the twin peak obtained with solution B (0.25%). The sum of the areas of all the peaks, other than the twin peak, is not greater than twice the area of the twin peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the twin peak in the chromatogram obtained with solution B.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 ml of each of the following 5 solutions in toluene R containing (A) 10 mg of Artenimol per ml, (B) 0.05 mg of Artenimol per ml, (C) 0.025 mg of Artenimol per ml, (D) 0.10 mg of Artenimol per ml, and (E) 0.10 mg of artenimol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/ sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Artenimol per ml, and solution (B) 1.0 mg of artenimol RS per ml.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

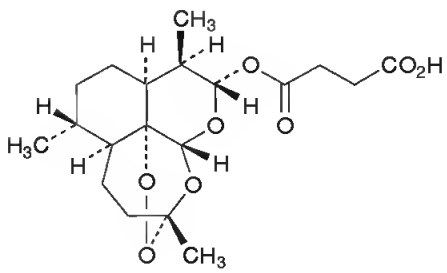
Measure the areas of the peak (twin-peak) responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{15}H_{22}O_5$ with reference to the dried substance.

B. Dissolve about 0.05 g of Artenimol, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $C_{15}H_{22}O_5$ in the substance being tested by comparison with artenimol RS, similarly and concurrently examined, and with reference to the dried substance.

ARTESUNATUM
ARTESUNATE

$C_{19}H_{28}O_8$



Relative molecular mass. 384.4

Chemical name. (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol, hydrogen succinate; CAS Reg. No. 182824-33-5.

Description. A fine, white crystalline powder.

Solubility. Very slightly soluble in water; very soluble in dichloromethane R; freely soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antimalarial drug.

Storage. Artesunate should be kept in a well-closed container, protected from light and stored in a cool place.

Requirements

Artesunate contains not less than **96.0%** and not more than the equivalent of **102.0%** of $C_{19}H_{28}O_8$ using Assay method A, and not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{19}H_{28}O_8$ using Assay method B, both calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artesunate RS or with the *reference spectrum* of artesunate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 5 volumes of ethyl acetate R and 95 volumes of toluene R as the mobile phase. Apply separately to the plate 2 µl of the following 2 solutions in toluene R containing (A) 0.10 mg of Artesunate per ml, and (B) 0.10 mg of artesunate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/methanol TS, and heat the plate to 120 °C for 5 minutes. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 0.1 g of Artesunate in 40 ml of dehydrated ethanol R, shake, and filter. To half of the filtrate (keep the remaining filtrate for test D) add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a light red-violet colour is produced.

D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish, add 1 drop of vanillin/sulfuric acid TS1; a reddish-brown colour is produced.

Melting range. 132–135 °C.

Specific optical rotation. Use a 10 mg/ml solution in dichloromethane R;

$[\alpha]_D^{20\,°C} = +4.5°$ to $+6.5°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 2 g of Artesunate; the water content is not more than 5 mg/g.

pH value. pH of an aqueous suspension containing 10 mg/g, 3.5–4.5.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (1.0%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice

the area of the principal peak obtained with solution C (2.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 48 volumes of light petroleum R1, 36 volumes of ethyl acetate R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 3 solutions in dichloromethane R containing (A) 5.0 mg of Artesunate per ml, (B) 0.05 mg of Artesunate per ml, and (C) 0.025 mg of Artesunate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/ sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (1.0%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (12.5 cm × 3.5 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of equal volumes of acetonitrile R and buffer pH 3.0 (dissolve 1.36 g of potassium dihydrogen phosphate R in 1000 ml of water and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).

Prepare the following solutions in acetonitrile R: solution (A) 4.0 mg of Artesunate per ml; solution (B) 4.0 mg of artesunate RS per ml; and for solution (C) dilute solution A to obtain a concentration equivalent to 0.04 mg of Artesunate per ml.

Operate with a flow rate of 0.6 ml per minute. Maintain the column temperature at 30 °C and use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

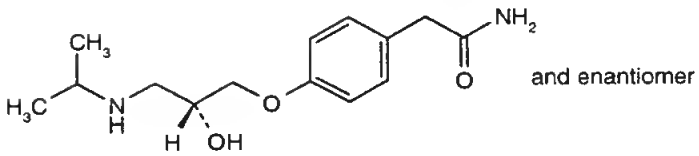
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{19}H_{28}O_8$ with reference to the anhydrous substance.

B. Dissolve about 0.25 g of Artesunate, accurately weighed, in 25 ml of neutralized ethanol TS and titrate with sodium hydroxide (0.05 mol/l) VS, using 2 drops of phenolphthalein/ethanol TS as indicator.

Each ml of sodium hydroxide (0.05 mol/l) VS is equivalent to 19.22 mg of $C_{19}H_{28}O_8$.

ATENOLOLUM

ATENOLOL



$C_{14}H_{22}N_2O_3$

Relative molecular mass. 266.3

Chemical name. 2-[*p*-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide (racemate); CAS Reg. No. 29122-68-7.

Description. A white or almost white powder.

Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in dichloromethane R.

Category. Cardiovascular agent; β-adrenoreceptor blocking agent.

Storage. Atenolol should be kept in a tightly closed container.

Requirements

Atenolol contains not less than **99.0%** and not more than **101.0%** of $C_{14}H_{22}N_2O_3$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with

the spectrum obtained from atenolol RS or with the *reference spectrum* of atenolol.

B. The absorption spectrum of a 0.10 mg/ml solution in methanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 275 nm and 282 nm. The ratio of the absorbance at 275 nm to that at 282 nm is between 1.15 and 1.20.

C. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 99 volumes of methanol R and 1 volume of ammonia (~260g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 10 mg of Atenolol per ml, and (B) 10 mg of atenolol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

D. Melting temperature, about 154 °C.

Chlorides. Dissolve 0.25 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 1.0 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (15 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). Prepare the following solution to be used as the mobile phase: dissolve 1.0 g of sodium octanesulfonate R and 0.4 g of tetrabutylammonium hydrogen sulfate R in 1000 ml of a mixture of 80 volumes of a 3.4 mg/ml solution of potassium dihydrogen phosphate R, the pH of the solution adjusted to 3.0 with phosphoric acid (~1440 g/l), 18 volumes of methanol R, and 2 volumes of tetrahydrofuran R.

Prepare the following solutions: for solution (A) dissolve 10 mg of Atenolol in 5 ml of mobile phase; for solution (B) dissolve 0.05 g of Atenolol in 0.10 ml of dimethyl sulfoxide R, if necessary applying gentle heat by placing the flask in a water-bath for a few seconds, and dilute with sufficient mobile phase to produce 25 ml; for solution (C) dilute 0.5 ml of solution A with sufficient mobile phase to produce 100 ml; and for solution (D) dissolve 0.05 g of atenolol for column validation RS in 0.10 ml of dimethyl sulfoxide R, if necessary applying

gentle heat by placing the flask in a water-bath for a few seconds, and dilute with sufficient mobile phase to produce 25 ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 226 nm.

Inject 10 µl of solution C. Adjust the sensitivity of the system so that the height of the principal peak is at least 50% of the full scale of the recorder.

Inject 10 µl of solution D. The tracing obtained is similar to that of the specimen chromatogram provided with atenolol for column validation RS, where the peak due to the bis-ether precedes and is separated from the tertiary amine which normally appears as a doublet. If necessary, adjust the concentration of sodium octanesulfonate R in the mobile phase: a higher concentration would increase the retention time of the tertiary amine.

Inject alternately 10 µl each of solutions A and C. Continue the recording of the chromatogram for four times the retention time of the principal peak.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than that of the principal peak obtained with solution C (0.5%). Disregard any peak with an area less than 0.1 times that of the principal peak obtained with solution C. If the content of bisether in Atenolol is greater than 0.15%, repeat the chromatography with 10 ml of solution B to confirm its compliance.

Assay. Dissolve about 0.2 g, accurately weighed, in 80 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

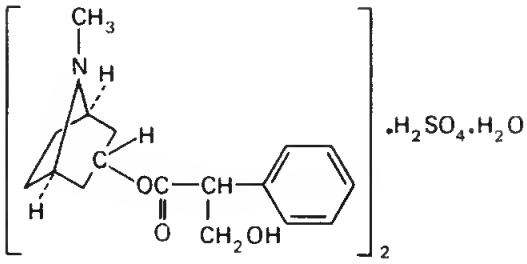
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.63 mg of $C_{14}H_{22}N_2O_3$.

ATROPINI SULFAS
ATROPINE SULFATE

Molecular formula. $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$

Relative molecular mass. 694.8

Graphic formula.



Chemical name. 1α*H*,5α*H*-Tropan-3α-ol (±)-tropate (ester) sulfate (2:1) (salt) monohydrate; (±)-*endo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl α-(hydroxymethyl) benzeneacetate sulfate (2:1) (salt) monohydrate; CAS Reg. No. 5908-99-6.

Description. Colourless crystal or a white, crystalline powder; odourless.

Solubility. Soluble in less than 1 part of water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R and benzene R.

Category. Cholinergic blocking agent (parasympatholytic).

Storage. Atropine sulfate should be kept in a tightly closed container, protected from light.

Additional information. Atropine sulfate is very poisonous; it effloresces in dry air; it is slowly affected by light.

Requirements

Definition. Atropine sulfate contains not less than 98.5% and not more than 101.0% of $(C_{17}H_{23}NO_3)_2,H_2SO_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or all 3 tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from atropine sulfate RS or with the *reference spectrum* of atropine sulfate.

B. Mix 1 mg with 5 drops of fuming nitric acid R and evaporate to dryness on a water-bath. To the cooled residue add 2 ml of acetone R and 3–4 drops of potassium hydroxide/methanol TS; a deep violet colour is produced.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

D. Dissolve 0.6 g in 30 ml of carbon-dioxide-free water R and add 2 ml of sodium hydroxide (~80 g/l) TS. Filter, wash the precipitate with water and dry at 100 °C. Melting temperature, about 116 °C (atropine base).

Optical rotation. Use a solution containing the equivalent of 0.10 g/ml of the dried substance, in a 200-mm tube; optical rotation = –0.50 to +0.10 ° (distinction from hyoscyamine).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 120 °C; it loses not less than 25 mg/g and not more than 40 mg/g.

Acidity. Dissolve 1.0 g in 20 ml of carbon-dioxide-free water R and titrate with sodium hydroxide (0.02 mol/l) VS, using methyl red/ethanol TS as indicator; not more than 0.3 ml is required to obtain the midpoint of the indicator (orange).

Readily oxidizable substances. To 10 ml of a 10 mg/ml solution add 0.1 ml of potassium permanganate (0.02 mol/l) VS; the colour is not completely discharged at the end of 3 minutes.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 6 volumes of ethylmethylketone R, 3 volumes of methanol R and 1 volume of ammonia (~100 g/l) TS as the mobile phase. Apply to the plate 10 µl of a solution in methanol R containing 12.5 mg/ml of the test substance. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2 and examine the chromatogram in daylight. No spot is obtained, other than the principal spot.

Assay. Dissolve about 0.6 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 67.68 mg of $(C_{17}H_{23}NO_3)_2,H_2SO_4$.

Additional requirements for Atropine sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 55.6 IU of endotoxin RS per mg.

Additional requirement for Atropine sulfate for sterile use

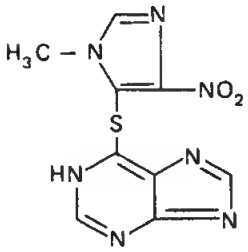
Complies with 3.2.1 Test for sterility of non-injectable preparations.

AZATHIOPRINUM
AZATHIOPRINE

Molecular formula. $C_9H_7N_7O_2S$

Relative molecular mass. 277.3

Graphic formula.



Chemical name. 6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine; CAS Reg. No. 446-86-6.

Description. A pale yellow powder; odourless.

Solubility. Practically insoluble in water; very slightly soluble in ethanol (~750 g/l) TS; sparingly soluble in dilute mineral acids; soluble in dilute solutions of alkali hydroxides.

Category. Immunosuppressive drug.

Storage. Azathioprine should be kept in a well-closed container, protected from light.

Additional information. Azathioprine decomposes in strong solutions of alkali hydroxides. CAUTION: Azathioprine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Azathioprine contains not less than 98.0% and not more than 101.5% of $C_9H_7N_7O_2S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from azathioprine RS or with the *reference spectrum* of azathioprine.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Heat 20 mg with 100 ml of water and filter. To 5 ml of the nitrate add 1 ml of hydrochloric acid (~420 g/l) TS, 10 mg of zinc R powder, and allow to stand for 5 minutes; the solution becomes yellow. Filter, cool in ice, add 0.1 ml of sodium nitrite (100 g/l) TS and 0.1 g of sulfamic acid R, and shake until the bubbles disappear. Add 1 ml of 2-naphthol TS1; a pale pink precipitate is produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 5 hours; it loses not more than 10 mg/g.

Acidity or alkalinity. Shake 0.5 g with 25 ml of water for 15 minutes, and filter; to 20 ml of the filtrate add 0.15 ml of methyl red/ethanol TS; not more than 0.10 ml of hydrochloric acid (0.02 mol/l) VS or 0.10 ml of sodium hydroxide (0.02 mol/l) VS is required to obtain the midpoint of the indicator (orange).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R3 (a precoated plate from a commercial source is suitable) and 1-butanol R saturated with ammonia (~100 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in ammonia (~100 g/l) TS containing (A) 10 mg of the test substance per ml, (B) 0.15 mg of the test substance per ml, and (C) 0.15 mg of azathioprine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 50 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 27.73 mg of $C_9H_7N_7O_2S$.

BACITRACINUM
BACITRACIN

Bacitracin (non-injectable)
Bacitracin, sterile

Chemical name. Bacitracin; CAS Reg. No. 1405-87-4.

Description. A white or pale brownish yellow powder; odourless or with a faint characteristic odour.

Solubility. Freely soluble in water, methanol R and ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Antiinfective drug.

Storage. Bacitracin should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 15 °C. If it is intended for parenteral administration, the container should be sterile and sealed so as to exclude microorganisms.

Labelling. The designation sterile Bacitracin indicates that the substance complies with the additional requirements for sterile Bacitracin and may be used for parenteral administration or for other sterile applications.

Additional information. Bacitracin is hygroscopic. Its solutions deteriorate rapidly at room temperature. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Bacitracin is a polypeptide produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis*. The main components are Bacitracin A, B_1, and B_2.

Bacitracin contains not less than 55 International Units per mg, calculated with reference to the dried substance.

Identity test

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 60 volumes of 1-butanol R, 10 volumes of water, 6 volumes of pyridine R, 15 volumes of glacial acetic acid R, and 5 volumes of ethanol (~750 g/l) TS as the mobile phase. Apply sepa-

rately to the plate 1 µl of each of 2 solutions in disodium edetate (10 g/l) TS containing (A) 6.0 mg of the test substance per ml and (B) 6.3 mg of bacitracin zinc RS per ml. A third spot (C) is made by applying 1 µl of each of solutions A and B at the same point of application, allowing to dry between the two loadings. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with triketohydrindene/pyridine/butanol TS, and heat it at 110 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight. The spots obtained with solution A correspond in position, appearance, and intensity with that obtained with solution B. A single spot is obtained with solution C.

Sulfated ash. Not more than 20 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

pH value. Shake 1.0 g with 10 ml of carbon-dioxide-free water R; the pH is between 5.5 and 7.5.

Bacitracin F and related substances. Prepare a solution containing 30 mg in 100 ml of sulfuric acid (0.05 mol/l) VS. The ratio of the absorbance at 290 nm to that at 252 nm is not greater than 0.15.

Assay. Dissolve 0.05 g, accurately weighed, in 5 ml of water and add 0.5 ml of hydrochloric acid (~70 g/l) TS and sufficient water to produce 100 ml. Allow to stand at room temperature for 30 minutes and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Micrococcus luteus* (NCTC 7743 or ATCC 10240) as the test organism, culture medium Cm1 with a final pH of either 7.0–7.1 or 6.5–6.6, sterile phosphate buffer TS of pH either 7.0 or 6.0, an appropriate concentration of bacitracin (usually 1–4 IU per ml), and an incubation temperature of either 35–39 °C or 32–35 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 55 IU per mg, calculated with reference to the dried substance.

Additional requirements for Bacitracin for sterile use

Storage. Sterile Bacitracin should be kept in a hermetically closed container, protected from light, and stored at a temperature not exceeding 15 °C.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of bacitracin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BACITRACINUM ZINCUM
BACITRACIN ZINC

Bacitracin zinc (non-injectable)
Bacitracin zinc, sterile

Chemical name. Bacitracin zinc; CAS Reg. No. 1405-89-6.

Description. A white or pale brownish yellow powder; odourless or with a faint characteristic odour.

Solubility. Soluble in 900 parts of water and in 500 parts of ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Antiinfective drug.

Storage. Bacitracin zinc should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 25 °C. If it is intended for parenteral administration, the container should be sterile and sealed so as to exclude microorganisms.

Labelling. The designation sterile Bacitracin zinc indicates that the substance complies with the additional requirements for sterile Bacitracin zinc and may be used for other sterile applications.

Additional information. Bacitracin zinc is hygroscopic.

Requirements

Definition. Bacitracin zinc is a zinc complex of bacitracin, a polypeptide produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis*. The main components are Bacitracin A, B_1 and B_2.

Bacitracin zinc contains not less than 55 International Units of bacitracin per mg, calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 60 volumes of 1-butanol R, 10 volumes of water, 6 volumes of pyridine R, 15 volumes of glacial acetic acid R, and 5 volumes of ethanol (~750 g/l) TS as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in disodium edetate (10 g/l) TS containing (A) 6.0 mg of the test substance per ml and (B) 6.0 mg of bacitracin zinc RS per ml. A third spot (C) is made by applying

1 µl of each of solutions A and B at the same point of application, allowing the plate to dry between the two loadings. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with triketohydrindene/pyridine/butanol TS, and heat it at 110 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight. The spots obtained with solution A correspond in position, appearance, and intensity with that obtained with solution B. A single spot is obtained with solution C.

B. Ignite 30 mg; dissolve half of the residue in 1.0 ml of hydrochloric acid (~70 g/l) TS and add 1.0 ml of potassium ferrocyanide (45 g/l) TS; a white precipitate is produced. Dissolve the remaining residue in 1.0 ml of sulfuric acid (~100 g/l) TS, add 0.05 ml of copper(II) sulfate (1 g/l) TS and 2.0 ml of ammonium mercurithiocyanate TS; a violet precipitate is produced.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

pH value. Shake 1.0 g with 10 ml of carbon-dioxide-free water R and filter; pH of the filtrate, 6.0–7.5.

Bacitracin F and related substances. Prepare a solution containing 30 mg in 100 ml of sulfuric acid (0.05 mol/l) VS. The ratio of the absorbance at 290 nm to that at 252 nm is not greater than 0.15.

Zinc. Dissolve 0.20 g in 5 ml of acetic acid (~60 g/l) TS and add 50 ml of water, 50 mg of xylenol orange indicator mixture R, and sufficient methenamine R to produce a red solution. Add 2.0 g of methenamine R in excess and titrate with disodium edetate (0.01 mol/l) VS until the colour changes to yellow. Each ml of disodium edetate (0.01 mol/l) VS is equivalent to 0.6537 mg of Zn; the zinc content is not less than 40 mg/g and not more than 60 mg/g, calculated with reference to the dried substance.

Assay. Suspend 0.05 g, accurately weighed, in 5 ml of water and add 0.5 ml of hydrochloric acid (~70 g/l) TS and sufficient water to produce 100 ml. Allow to stand at room temperature for 30 minutes and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Micrococcus luteus* (NCTC 7743 or ATCC 10240) as the test organism, culture medium Cm1 with a final pH of either 7.0–7.1 or 6.5–6.6, sterile phosphate buffer TS of pH either 7.0 or 6.0, an appropriate concentration of bacitracin (usually 1–4 IU per ml), and an incubation temperature of either 35–39 °C or 32–35 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 55 IU of bacitracin per mg, calculated with reference to the dried substance.

Additional requirements for Bacitracin zinc for sterile use

Storage. Sterile Bacitracin zinc should be kept in a hermetically closed container, protected from light, and stored at a temperature not exceeding 25 °C.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure. Dissolve the test substance in peptone (1 g/l) TS1 to which disodium edetate R has been added. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BARII SULFAS
BARIUM SULFATE

Molecular formula. $BaSO_4$

Relative molecular mass. 233.4

Chemical name. Barium sulfate (1:1); CAS Reg. No. 7727-43-7.

Description. A white, heavy, fine powder; free from grittiness; odourless.

Solubility. Practically insoluble in water and in organic solvents; very slightly soluble in acids and in solutions of alkali hydroxides.

Category. Radiocontrast medium.

Storage. Barium sulfate should be kept in a well-closed container.

Additional information. Barium sulfate is inclined to caking.

Requirements

Identity tests

A. Boil 0.2 g in a solution of 5.0 g of sodium carbonate R dissolved in 5 ml of water for 5 minutes, then add 10 ml of water and filter (keep the precipitate for test B). To 5 ml of the filtrate add 5 ml of hydrochloric acid (~70 g/l) TS; this solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

B. Wash the precipitate from test A with 3 successive small quantities of water. To the residue add 5 ml of hydrochloric acid (~70 g/l) TS, filter, and to the filtrate add 0.3 ml of sulfuric acid (~100 g/l) TS; a white precipitate is produced which is insoluble in sodium hydroxide (~80 g/l) TS.

Sedimentation. Place 5.0 g, previously sifted, in a glass-stoppered 50-ml graduated cylinder, having the 50-ml graduation mark 11–14 cm from the base. Add sufficient water to produce 50 ml, shake for 5 minutes and allow to stand for 15 minutes; the barium sulfate does not settle below the 15-ml graduation mark.

Heavy metals. For the preparation of the test solution boil 4 g with 6 ml of acetic acid (~60 g/l) PbTS and 44 ml of water for 10 minutes, filter, allow to cool and dilute to 50 ml with water. Determine the heavy metals content in 25 ml of the filtrate as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 10 µg/g.

Arsenic. Transfer 0.5 g to a long-necked combustion flask, add 30 ml of water and 2 ml of nitric acid (~1000 g/l) TS, insert a small funnel into the neck of the flasks and heat in an inclined position on a water-bath for 2 hours. Allow to cool, adjust to the original volume with water, and filter. Wash the residue three times with 5 ml of water, combine the filtrate and washings, add 1 ml of sulfuric acid (~1760 g/l) TS, and evaporate on a water-bath until white fumes are evolved. Dissolve the residue in 10 ml of sulfuric acid (~100 g/l) TS, add 10 ml of water, and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Soluble barium salts. Boil 10 g with 20 ml of water and 30 ml of acetic acid (~60 g/l) TS for 5 minutes, filter, allow to cool and dilute to 50 ml with water. To a 10-ml portion of this solution add 1 ml of sulfuric acid (~100 g/l) TS and to a second 10-ml portion add 1 ml of water. When compared after 1 hour, the two solutions remain equally clear.

Phosphates. To 1.0 g add 3 ml of nitric acid (~130 g/l) TS and 7 ml of water and heat on a water-bath for 5 minutes. Filter and dilute the filtrate to 10 ml with water. Add 5 ml of ammonium molybdate/vanadate TS and allow to stand for 5 minutes; any yellow colour produced is not more intense than that of a reference solution prepared similarly using 10 ml of phosphate standard (5 µg/ml) TS.

Oxidizable sulfur compounds. Shake 1.0 g with 5 ml of water for 30 seconds and filter. To the filtrate add 0.1 ml of starch TS, 0.1 g of potassium iodide R, 1 ml of freshly prepared potassium iodate (3.6 mg/l) TS, and 1 ml of hydrochloric acid (1 mol/l) VS, and shake well; the colour produced is more intense than that of a solution prepared in a similar way, but omitting the potassium iodate.

Acid-soluble substances. Boil 5 g with 15 ml of acetic acid (~300 g/l) TS and 10 ml of water for 5 minutes. Filter, evaporate the filtrate to dryness on a water-bath, and dry to constant weight at 105 °C; the residue weighs not more than 15 mg.

Loss on ignition. Ignite 1.0 g at 600 °C; it loses not more than 20 mg/g.

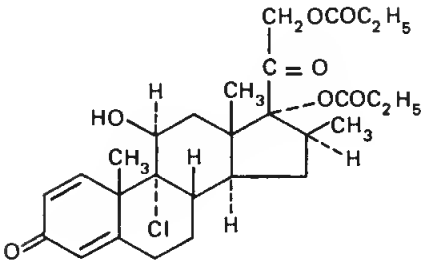
Acidity or alkalinity. Heat 5.0 g with 20 ml of carbon-dioxide-free water R on a water-bath for 5 minutes and filter. To 10 ml of the filtrate add 0.05 ml of bromothymol blue/ethanol TS; not more than 0.5 ml of hydrochloric acid (0.01 mol/l) VS or 0.5 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS is required to obtain the midpoint of the indicator (green).

BECLOMETASONI DIPROPIONAS
BECLOMETASONE DIPROPIONATE

Molecular formula. $C_{28}H_{37}ClO_7$

Relative molecular mass. 521.0

Graphic formula.



Chemical name. 9-Chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate; 9-chloro-11β-hydroxy-16β-methyl-17,21-bis(1-oxopropoxy)pregna-1,4-diene-3,20-dione; CAS Reg. No. 5534-09-8.

Description. A white to creamy white powder; odourless.

Solubility. Practically insoluble in water; soluble in 60 parts of ethanol (~750 g/l) TS.

Category. Antiasthmatic drug.

Storage. Beclometasone dipropionate should be kept in a well-closed container, protected from light.

Requirements

Definition. Beclometasone dipropionate contains not less than 96.0% and not more than 104.0% of $C_{28}H_{37}ClO_7$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from beclometasone dipropionate RS or with the *reference spectrum* of beclometasone dipropionate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use a mixture of 4 volumes of cyclohexane R and 1 volume of toluene R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of beclometasone dipropionate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat it at 120 °C for 15 minutes, spray the hot plate with sulfuric acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +88$ to $+94°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R, and 0.2 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of 2 solu-

tions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated and heat at 105 °C for 10 minutes; allow it to cool, spray it with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

- The solutions must be protected from light throughout the assay.

Dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Dilute 20 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS, and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. Calculate the amount of $C_{28}H_{37}ClO_7$ in the substance being tested by comparison with beclometasone dipropionate RS, similarly and concurrently examined.

BENTONITUM
BENTONITE

Chemical name. Bentonite; CAS Reg. No. 1302-78-9.

Description. A greyish white to cream-coloured, very fine, homogeneous powder; odourless.

Solubility. Practically insoluble in water and most organic solvents; when added to water it swells to approximately 12 times its volume.

Category. Suspending agent; viscosity-increasing agent.

Storage. Bentonite should be kept in a tightly closed container.

Additional information. Bentonite may also contain calcium, magnesium, and iron. Attention should be paid to the microbiological quality since Bentonite is of mineral origin.

Requirements

Definition. Bentonite is a natural, colloidal, hydrated aluminium silicate.

Identity tests

A. In a metal crucible fuse 0.5 g with 0.4 g of anhydrous sodium carbonate R. Add hot water to the residue and filter. (Keep the filtrate for test B.) Add a few drops of hydrochloric acid (~420 g/l) TS to the residue on the filter, dilute to 5 ml with water, and filter. To 2 ml of the filtrate add 2 ml of ammonium chloride (100 g/l) TS and 2 ml of ammonia (~100 g/l) TS; a white, gelatinous precipitate is produced which is soluble in hydrochloric acid (~420 g/l) TS, acetic acid (~300 g/l) TS, and sodium hydroxide (~80 g/l) TS, but insoluble in ammonia (~260 g/l) TS.

B. Acidify the filtrate from test A with hydrochloric acid (~420 g/l) TS and evaporate to dryness. Heat the residue with a mixture of 10 mg of calcium fluoride R and a few drops of sulfuric acid (~1760 g/l) TS; a gas is evolved which, if bubbled into water, gives a white precipitate.

Loss on drying. Dry to constant mass at 105 °C; it loses not less than 50 mg/g and not more than 150 mg/g.

Alkalinity. Shake 2 g with 100 ml of carbon-dioxide-free water R for 5 minutes using a stoppered flask. To 5 ml of the suspension add 0.1 ml of thymolphthalein/ethanol TS; a bluish colour is produced. Add 0.1 ml of hydrochloric acid (0.1 mol/l) VS and allow to stand for 5 minutes; the solution becomes colourless.

Sedimentation volume. Mix 6 g with 0.3 g of freshly calcined light magnesium oxide R and add progressively 200 ml of water. Shake for 1 hour, place 100 ml of the suspension in a graduated cylinder, and allow to stand for 24 hours; the volume of the supernatant liquid is not larger than 2 ml.

Swelling power. Transfer 100 ml of sodium laurilsulfate (10 g/l) TS to a glass-stoppered cylinder with a volume of 100 ml and add, in 20 portions, 2 g of Bentonite at intervals of at least 2 minutes, permitting each portion to settle before adding the next. Allow to stand for 2 hours; the apparent volume of the sediment at the bottom of the cylinder is not less than 22 ml.

Fineness of powder. Triturate 2 g in a mortar with 20 ml of water. Allow to swell, disperse evenly with a pestle, and dilute with water to 100 ml. Pour the

suspension through a sieve with the nominal aperture size of 75 µm (sieve no. 75), and wash the sieve thoroughly with water; no grit is felt when the fingers are rubbed over the wire mesh of the sieve.

BENZALKONII CHLORIDUM
BENZALKONIUM CHLORIDE

Chemical name. Alkylbenzyldimethylammonium chloride; alkyldimethyl (phenylmethyl)ammonium chloride; CAS Reg. No. 8001-54-5.

Description. A white or yellowish white powder, thick gel, or gelatinous pieces; odourless or a slight aromatic odour.

Solubility. Very soluble in water and ethanol (~750 g/l) TS; freely soluble in acetone R; practically insoluble in ether R.

Category. Antimicrobial preservative; surfactant.

Storage. Benzalkonium chloride should be kept in a tightly closed container, protected from light.

Additional information. Benzalkonium chloride is hygroscopic.

Requirements

Definition. Benzalkonium chloride is a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C_8 to C_{18}.

Benzalkonium chloride contains not less than **95.0%** and not more than the equivalent of **104.0%** of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$ (relative molecular mass 354.0) and with reference to the anhydrous substance.

Identity tests

A. Shake a solution of 0.1 g in 100 ml of water; it foams strongly.

B. To 5 ml of sodium hydroxide (~80 g/l) TS add 0.1 ml of bromophenol blue TS and 5 ml of chloroform R and shake; the chloroform layer is colourless. Dissolve 10 mg in 1 ml of carbon-dioxide-free water R, add 0.1 ml to the solution above, and shake; the chloroform layer becomes blue.

C. A solution of 10 mg/ml in a mixture of equal volumes of water and ethanol (~750 g/l) TS yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 20 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.1 g; the water content is not more than 150 mg/g.

Ammonium compounds. Dissolve 0.1 g in 5 ml of water, add 3 ml of sodium hydroxide (1 mol/l) VS, and heat to boiling. Place a moistened strip of red litmus paper R over the solution; no blue colour appears on the paper.

Assay. Dissolve about 2 g, accurately weighed, in sufficient water to produce 100 ml. Transfer 25 ml to a separating funnel, add 25 ml of chloroform R, 10 ml of sodium hydroxide (0.1 mol/l) VS, and 10 ml of a freshly prepared solution of 50 mg of potassium iodide R per ml. Shake well, allow to separate, and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of chloroform R and discard the chloroform layers. To the aqueous layer add 40 ml of hydrochloric acid (~420 g/l) TS, allow to cool, and titrate with potassium iodate (0.05 mol/l) VS until the deep brown colour is discharged. Add 2 ml of chloroform R and continue the titration, shaking vigorously, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 10 ml of the freshly prepared potassium iodide solution as described above, 20 ml of water, and 40 ml of hydrochloric acid (~420 g/l) TS and make any necessary corrections.

Each ml of potassium iodate (0.05 mol/l) VS is equivalent to 35.40 mg of $C_{22}H_{40}ClN$.

BENZATHINI BENZYLPENICILLINUM

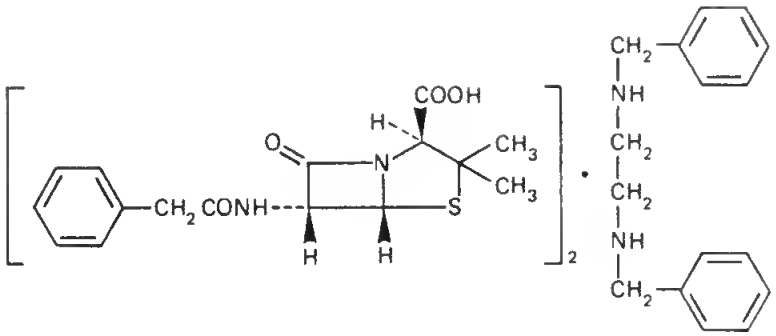
BENZATHINE BENZYLPENICILLIN

Benzathine benzylpenicillin (non-injectable)
Benzathine benzylpenicillin, sterile

Molecular formula. $(C_{16}H_{18}N_2O_4S)_2,C_{16}H_{20}N_2$ (anhydrous)

Relative molecular mass. 909.1 (anhydrous)

Graphic formula.



Chemical name. *N,N'*-Dibenzylethylenediamine compound with (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1:2); *N,N'*-bis(phenylmethyl)-1,2-ethanediamine compound with [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1:2); *N,N'*-dibenzylethylenediamine salt of benzylpenicillin; CAS Reg. No. 1538-09-6 (anhydrous).

Other name. Penicillin G benzathine.

Description. A white powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Benzathine benzylpenicillin should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 30 °C.

Labelling. The designation sterile Benzathine benzylpenicillin indicates that the substance complies with the additional requirements for sterile Benzathine benzylpenicillin and may be used for parenteral administration or for other sterile applications.

Additional information. Benzathine benzylpenicillin contains a variable amount of water of crystallization.

Requirements

Definition. Benzathine benzylpenicillin contains not less than 96.0% and not more than 100.5% of total penicillins calculated as $(C_{16}H_{18}N_2O_4S)_2,C_{16}H_{20}N_2$, and not less than 24.0% and not more than 27.0% of $C_{16}H_{20}N_2$, both calculated with reference to the anhydrous substance.

Identity tests

A. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is almost colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains almost colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is almost colourless and after a few minutes the colour changes to yellow-brown. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

B. Shake 0.1 g with 2 ml of sodium hydroxide (1 mol/l) VS for 2 minutes, extract the mixture with 2 quantities, each of 3 ml of ether R, evaporate the combined extracts, and dissolve the residue in 1 ml of ethanol (~375 g/l) TS. Add 5 ml of trinitrophenol (7 g/l) TS, heat at 90 °C for 5 minutes, and allow to cool slowly. Collect the precipitate and recrystallize it from hot ethanol (~150 g/l) TS that contains 10 mg/ml of trinitrophenol R; melting temperature, about 214 °C (picrate).

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not less than 50 mg/g and not more than 80 mg/g.

pH value. pH of a saturated solution containing about 0.05 g in 10 ml of carbon-dioxide-free water R, 5.0-7.5.

Assay

A. *For total penicillins.* Dissolve about 0.065 g, accurately weighed, in 10 ml of dimethylformamide R and dilute with sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place it in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $(C_{16}H_{18}N_2O_4S)_2,C_{16}H_{20}N_2$ in the substance being tested by comparison with 0.050 g of benzylpenicillin sodium RS similarly and concurrently examined, taking into account that each mg of benzylpenicillin sodium RS ($C_{16}H_{17}N_2O_4S$) is equivalent to 1.275 mg of $(C_{16}H_{18}N_2O_4S)_2,C_{16}H_{20}N_2$. In an adequately calibrated spectrophotometer, the absorbance of the reference solution should be 0.62 ± 0.03.

B. *For* $C_{16}H_{20}N_2$. To about 1 g, accurately weighed, add 30 ml of sodium chloride (400 g/l) TS and 10 ml of sodium hydroxide (~150 g/l) TS, shake well, and extract with four quantities, each of 50 ml of ether R. Wash the combined extracts with three quantities, each of 10 ml of water, extract the combined washings with 25 ml of ether R, and add the extract to the main ether solution. Evaporate the ether solution to a low bulk, add 2 ml of dehydrated ethanol R and evaporate to dryness. To the residue add 50 ml of glacial acetic acid R and titrate with perchloric acid (0.1 mol/l) VS, using 1 ml of 1-naphtholbenzein/acetic acid TS as indicator. Repeat the operation without the substance being examined; the difference between the titrations represents the amount of perchloric acid required to neutralize the liberated base. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 12.02 mg of $C_{16}H_{20}N_2$.

Additional requirements for Benzathine benzylpenicillin for sterile use

Storage. Sterile Benzathine benzylpenicillin should be kept in a hermetically closed container, protected from light.

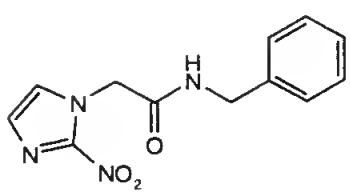
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

Additional requirements for Benzathine benzylpenicillin for parenteral use

Complies with the monograph for "Parenteral preparations".

BENZNIDAZOLUM
BENZNIDAZOLE



$C_{12}H_{12}N_4O_3$

Relative molecular mass. 260.3

Chemical name. *N*-Benzyl-2-nitroimidazole-1-acetamide; *N*-benzyl-2-nitro-1-imidazole-acetamide; CAS Reg. No. 22994-85-0.

Description. A yellowish powder; odourless or almost odourless.

Solubility. Practically insoluble in water; sparingly soluble in acetone R; slightly soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS.

Category. Antiprotozoal drug.

Storage. Benznidazole should be kept in a well-closed container, protected from light.

Requirements

Benznidazole contains not less than **98.5%** and not more than the equivalent of **101.5%** of $C_{12}H_{12}N_4O_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from benznidazole RS or with the *reference spectrum* of benznidazole.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Melting temperature, about 190 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 40 volumes of chloroform R, 40 volumes of ethyl acetate R, 15 volumes of methanol R, and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 20 µl of each of 3 solutions in acetone R containing (A) 25 mg of Benznidazole per ml, (B) 25 mg of benznidazole RS per ml, and (C) 125 µg of benznidazole RS per ml. After removing the plate from the chro-

matographic chamber, allow it to dry in air until the solvents have evaporated, and heat at 110 °C for 10 minutes. Allow it to cool and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

Assay. Dissolve about 0.2 g, accurately weighed, in 75 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

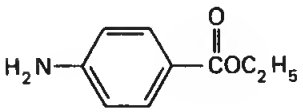
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.03 mg of $C_{12}H_{12}N_4O_3$.

BENZOCAINUM
BENZOCAINE

Molecular formula. $C_9H_{11}NO_2$

Relative molecular mass. 165.2

Graphic formula.



Chemical name. Ethyl *p*-aminobenzoate; ethyl 4-aminobenzoate; CAS Reg. No. 94-09.7.

Other name. Ethyl aminobenzoate.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; soluble in 6 parts of ethanol (~750 g/l) TS and 5.5 parts of ether R.

Category. Local anaesthetic.

Storage. Benzocaine should be kept in a well-closed container, protected from light.

Additional information. Benzocaine causes local numbness after being placed on the tongue.

Requirements

Definition. Benzocaine contains not less than 98.0% and not more than 101.0% of $C_9H_{11}NO_2$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.10 g in 5 ml of water, add 3 drops of hydrochloric acid (~70 g/l) TS and 5 drops of iodine TS; a brown precipitate is produced.

B. Heat 0.05 g with 2 drops of acetic acid (~300 g/l) TS and 4 drops of sulfuric acid (~1760 g/l) TS; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

C. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing an orange-red precipitate.

Melting range. 88–92 °C.

Heavy metals. Use 1.0 g and ethanol (~750 g/l) TS for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 10 µg/g.

Solution in ethanol. A solution of 1.0 g in 10 ml of ethanol (~750 g/l) TS is clear and colourless.

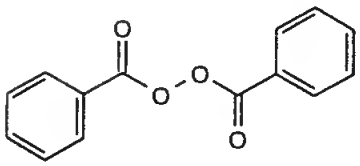
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R or phosphorus pentoxide R; it loses not more than 10 mg/g.

Acidity or alkalinity. To a solution of 0.5 g in 10 ml of neutralized ethanol TS add 10 ml of carbon-dioxide-free water R and 2 drops of phenolphthalein/ethanol TS; no pink colour is produced. Add 0.5 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS; a pink colour is produced.

Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.3 g, accurately weighed, dissolved in 50 ml of hydrochloric acid (~70 g/l) TS and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

BENZOYLIS PEROXIDUM CUM AQUA
HYDROUS BENZOYL PEROXIDE



$C_{14}H_{10}O_4$, xH_2O

Relative molecular mass. 242.2 (anhydrous)

Chemical name. Dibenzoyl peroxide; CAS Reg. No. 94-36-0.

Description. A white, amorphous or granular powder.

Solubility. Practically insoluble in water; soluble in acetone R; soluble in dichloromethane R with separation of water; slightly soluble in ethanol (~750 g/l) TS.

Category. Keratolytic agent.

Storage. Hydrous Benzoyl peroxide should be kept in a container that has been treated to reduce static discharge and that has a device for the release of excess pressure. Store at a temperature between 2 and 8 °C, protected from light.

Additional information. *CAUTION*: Hydrous Benzoyl peroxide may explode at temperatures higher than 60 °C or if its water content is too low. It may burst into flame in the presence of reducing substances. Unused material must not be returned to the original container but destroyed by treating with sodium hydroxide (~80 g/l) TS to a point where no iodine is liberated after acidifying with hydrochloric acid (~70 g/l) TS and adding a crystal of potassium iodide R.

Hydrous Benzoyl peroxide loses water rapidly on exposure to air. It must be handled with care, avoiding contact with the skin and mucous membranes and inhalation of airborne particles.

Requirements

Hydrous Benzoyl peroxide contains not less than **70.0%** and not more than **77.0%** of $C_{14}H_{10}O_4$, and not less than **20.0%** of water.

Note: Before carrying out any tests, thoroughly mix the entire sample.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of benzoyl peroxide.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 50 volumes of toluene R, 2 volumes of dichloromethane R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 ml of each of 2 solutions in methanol R containing (A) 10.0 mg of Hydrous Benzoyl peroxide per ml, and (B) a solution of hydrous benzoyl peroxide R containing the equivalent of 10.0 mg of benzoyl peroxide per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve about 25 mg in 2 ml of acetone R, add 1 ml of diethylphenylenediamine sulfate TS, and mix; a red colour is produced which turns rapidly to dark violet within 5 minutes.

D. To 1 g add 5 ml of ethanol (~750 g/l) TS, 5 ml of sodium hydroxide (~80 g/l) TS, and 10 ml of water. Boil the mixture under a reflux condenser for 20 minutes and cool. To 1 ml of the resulting solution add 0.5 ml of ferric chloride (65 g/l) TS; a dull yellow precipitate is produced which is soluble in ether R.

Chlorides. Dissolve a quantity containing the equivalent of 0.5 g of anhydrous Benzoyl peroxide in 15 ml of acetone R. Add, while stirring, 50 ml of nitric acid (0.05 mol/l) VS, allow to stand for 10 minutes, and filter. Wash the residue with two quantities, each of 10 ml, of nitric acid (0.05 mol/l) VS, combining the filtrate and the washings. Dilute this solution to 100 ml with nitric acid (0.05 mol/l) VS. Using 2.5 ml of this solution, proceed as described under 2.2.1 Limit test for chlorides; the chloride content does not exceed 4 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 5.0 ml of solution A as prepared below under "Assay". Add 3 ml of a solution containing 0.10 g of potassium iodide R in dimethylformamide R. Stir for 5 minutes before starting the titration. Repeat the procedure using 5 ml of dimethylformamide R in place of solution A and make any necessary corrections. Calculate the content of water as a percentage.

Acidity. Dissolve a quantity containing the equivalent of 1.0 g of anhydrous Benzoyl peroxide in 25 ml of acetone R, add 75 ml of water, and filter. Wash

the residue with two quantities of 10 ml of water. Combine the filtrate and washings, and titrate with sodium hydroxide (0.1 mol/l) VS, using 0.25 ml of phenolphthalein/ethanol TS as indicator, until the change in colour is observed. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required; not more than 1.25 ml of sodium hydroxide (0.1 mol/l) VS.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 40 volumes of light petroleum R1, 20 volumes of toluene R, 15 volumes of acetone R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 4 freshly prepared solutions in acetone R containing (A) a quantity equivalent to 40 mg of anhydrous Benzoyl peroxide per ml, (B) 0.4 mg of anhydrous Benzoyl peroxide per ml, (C) 0.6 mg of benzoic acid R per ml, and for solution (D) mix 0.4 ml of benzyl benzoate R with 5 ml of acetone R and dilute to 10 ml with the same solvent. To 1.0 ml of this solution add 1.0 ml of solution A and dilute to 10 ml with acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air for 20 minutes, and examine the chromatogram in ultraviolet light (254 nm).

Any spot corresponding to benzoic acid obtained with solution A is not more intense than that obtained with solution C (1.5%). Any spot obtained with solution A, other than the principal spot and the spot corresponding to benzoic acid, is not more intense than that obtained with solution B (1%). The test is not valid unless the chromatogram obtained with solution D shows two clearly separated principal spots.

Assay. Immediately before testing dissolve 2.5 g in sufficient dimethylformamide R to produce 100 ml (*solution A*). To 5.0 ml of *solution A* add 20 ml of acetone R and 5 ml of potassium iodide (300 g/l) TS. Mix, allow to stand for 1 minute, and titrate with sodium thiosulfate (0.1 mol/l) VS until the solution is colourless. Repeat the procedure using 5 ml of dimethylformamide R in place of *solution A* and make any necessary corrections.

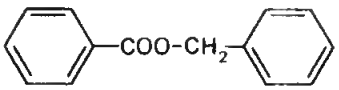
Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.

BENZYLIS BENZOAS
BENZYL BENZOATE

Molecular formula. $C_{14}H_{12}O_2$

Relative molecular mass. 212.3

Graphic formula.



Chemical name. Phenylmethyl benzoate; CAS Reg. No. 120-51-4.

Description. A clear, colourless, oily liquid; odour, faintly aromatic.

Miscibility. Practically immiscible with water and glycerol R; miscible with ethanol (~750 g/l) TS and ether R.

Category. Scabicide (topical use).

Storage. Benzyl benzoate should be kept in a tightly closed and well-filled container, protected from light.

Additional information. Benzyl benzoate may slowly decompose on contact with air.

Requirements

Definition. Benzyl benzoate contains not less than 98.0% and not more than 100.5% of $C_{14}H_{12}O_2$.

Identity tests
Boil 2 g with 25 ml of potassium hydroxide/ethanol TS2 for 10 minutes, evaporate the ethanol on a water-bath, cool, extract with 2 successive quantities, each of 15 ml of ether R, and proceed as follows:

A. Evaporate the ethereal layer on a water-bath; heat 1 drop of the oily liquid with 5 ml of sodium carbonate (50 g/l) TS and 1 ml of potassium permanganate (0.02 mol/l) VS; an odour of benzaldehyde is discernible.

B. To the aqueous layer add 10 ml of sulfuric acid (~100 g/l) TS; a white, crystalline precipitate is produced. Wash and dry the precipitate; melting temperature, about 123 °C (benzoic acid).

Congealing temperature. Not below 17.0 °C.

Refractive index. $n_D^{20} = 1.568 - 1.570$.

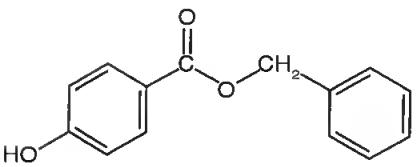
Mass density. $\rho_{20} = 1.116 - 1.120$ g/ml.

Chlorinated compounds. For the preparation of the test solution dissolve 0.30 g in 15 ml of ethanol (~750 g/l) TS and add 6 ml of sodium hydroxide (~80 g/l) TS. Warm the solution for 5 minutes on a water-bath. Cool, transfer to a comparison tube, and add 3 ml of nitric acid (~130 g/l) TS. For the reference solution transfer separately 2.0 ml of hydrochloric acid CITS and 4 ml of nitric acid (~130 g/l) TS to a comparison tube, and dilute to 25 ml with water. To both tubes add 0.5 ml of silver nitrate (40 g/l) TS. Stir immediately with a glass rod and set aside for 5 minutes, protected from direct sunlight. The opalescence produced from the test liquid is not stronger than that produced from the reference solution; not more than 0.33 mg/g of chlorine.

Acidity. Add 5 ml of the test liquid to 5 ml of neutralized ethanol TS, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, phenolphthalein/ethanol TS being used as indicator; not more than 0.3 ml is required to obtain the midpoint of the indicator (pink).

Assay. Add about 2.0 g, accurately weighed, to 40 ml of potassium hydroxide/ethanol (0.5 mol/l) VS and boil under reflux for 1 hour. Cool, and titrate with hydrochloric acid (0.5 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the test liquid being examined and make any necessary corrections. Each ml of potassium hydroxide/ethanol (0.5 mol/l) VS is equivalent to 106.1 mg of $C_{14}H_{12}O_2$.

BENZYLIS HYDROXYBENZOAS
BENZYL HYDROXYBENZOATE



$C_{14}H_{12}O_3$

Relative molecular mass. 228.3

Chemical name. Benzyl *p*-hydroxybenzoate; phenylmethyl 4-hydroxybenzoate; CAS Reg. No. 94-18-8.

Description. A white to creamy white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS and ether R; dissolves in solutions of alkali hydroxides.

Category. Antimicrobial preservative.

Storage. Benzyl hydroxybenzoate should be kept in a well-closed container.

Requirements

Benzyl hydroxybenzoate contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{14}H_{12}O_3$.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in ethanol (~750 g/l) TS, when observed between 230 nm and 350 nm, exhibits a maximum at about 260 nm; the absorbance of a 1-cm layer at this wavelength is about 0.76.

B. Dissolve 0.1 g in 2 ml of ethanol (~750 g/l) TS, boil, and add 0.5 ml of mercury/nitric acid TS; a precipitate gradually separates and the supernatant liquid becomes red.

C. Melting temperature, about 112 °C.

Sulfated ash. Not more than 1.0 mg/g.

Acidity. Dissolve 0.2 g in 10 ml of ethanol (~375 g/l) TS previously neutralized using methyl red/ethanol TS. Titrate with sodium hydroxide (0.1 mol/l) VS; not more than 0.1 ml is required to obtain the midpoint of the indicator (orange).

Assay. To about 0.12 g, accurately weighed, add 20 ml of sodium hydroxide (~80 g/l) TS, and boil gently under reflux for 30 minutes. Cool, and extract with three quantities, each of 20 ml, of dichloroethane R. Wash the combined extracts with 20 ml of sodium hydroxide (0.1 mol/l) VS and add the wash liquids to the main aqueous phase, discarding the organic phase. To the aqueous solution add 25 ml of potassium bromate (0.0333 mol/l) VS, 6 ml of potassium bromide (100 g/l) TS, and 10 ml of hydrochloric acid (~420 g/l) TS and immediately stopper the flask. Shake for 15 minutes and allow to stand for 15 minutes. Add 25 ml of potassium iodide (100 g/l) TS and shake vigorously. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, added towards the end of the titration. Repeat the procedure without the Benzyl hydroxybenzoate being examined and make any necessary corrections. The volume of potassium bromate (0.0333 mol/l) VS used is equivalent to half of the volume of sodium thiosulfate (0.1 mol/l) VS required for the titration.

Each ml of potassium bromate (0.0333 mol/l) VS is equivalent to 7.608 mg of $C_{14}H_{12}O_3$.

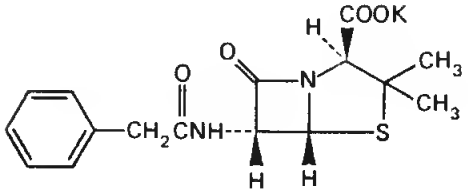
BENZYLPENICILLINUM KALICUM
BENZYLPENICILLIN POTASSIUM

Benzylpenicillin potassium (non-injectable)
Benzylpenicillin potassium, sterile

Molecular formula. $C_{16}H_{17}KN_2O_4S$

Relative molecular mass. 372.5

Graphic formula.



Chemical name. Potassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; potassium [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; CAS Reg. No. 113-98-4.

Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.

Solubility. Very soluble in water; practically insoluble in ether R.

Category. Antibiotic.

Storage. Benzylpenicillin potassium should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 25 °C.

Labelling. The designation sterile Benzylpenicillin potassium indicates that the substance complies with the additional requirements for sterile Benzylpenicillin potassium and may be used for parenteral administration or for other sterile applications.

Additional information. Benzylpenicillin potassium is moderately hygroscopic; it is readily decomposed by acids, alkalis and oxidizing agents. Even in the absence of light, Benzylpenicillin potassium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Benzylpenicillin potassium contains not less than 96.0% and not more than 102.0% of $C_{16}H_{17}KN_2O_4S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from benzylpenicillin potassium RS or with the *reference spectrum* of benzylpenicillin potassium.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is brownish yellow. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

C. Ignite a small quantity, dissolve the residue in water and filter; on addition of 2 ml of sodium hydroxide (~80 g/l) TS to the filtrate it yields the reaction described under 2.1 General identification tests, as characteristic of potassium.

Specific optical rotation. Use a 20 mg/ml solution; $[\alpha]_D^{20\,°C} = +270$ to $+300°$.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 5.0–7.5.

Light-absorbing impurities. Using a freshly prepared 1.9 mg/ml solution in water, measure the absorbances of a 1-cm layer at 280 nm and at 325 nm; the absorbance at each of these wavelengths does not exceed 0.10.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{17}KN_2O_4S$ in the substance being tested by comparison with benzylpenicillin sodium RS similarly and concurrently examined, taking into account that each mg of benzylpenicillin sodium RS ($C_{16}H_{17}N_2NaO_4S$) is equivalent to 1.045 mg of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$). In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.62 ± 0.03.

Additional requirements for Benzylpenicillin potassium for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BENZYLPENICILLINUM NATRICUM

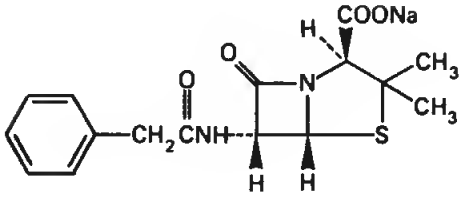
BENZYLPENICILLIN SODIUM

Benzylpenicillin sodium (non-injectable)
Benzylpenicillin sodium, sterile

Molecular formula. $C_{16}H_{17}N_2NaO_4S$

Relative molecular mass. 356.4

Graphic formula.



Chemical name. Sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; sodium [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylate; CAS Reg. No. 69-57-8.

Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.

Solubility. Soluble in about 0.5 part of water; practically insoluble in ether R.

Category. Antibiotic.

Storage. Benzylpenicillin sodium should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 25 °C.

Labelling. The designation sterile Benzylpenicillin sodium indicates that the substance complies with the additional requirements for sterile Benzylpenicillin sodium and may be used for parenteral administration or for other sterile applications.

Additional information. Benzylpenicillin sodium is hygroscopic; it is readily decomposed by acid, alkalis and oxidizing agents. Even in the absence of light, Benzylpenicillin sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Benzylpenicillin sodium contains not less than 96.0% and not more than 102.0% of $C_{16}H_{17}N_2NaO_4S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from benzylpenicillin sodium RS or with the *reference spectrum* of benzylpenicillin sodium.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is brownish yellow. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

C. When tested for sodium as described under 2.1 General identification tests yields the characteristic reaction. If reaction B is to be used, ignite a small quantity and dissolve the residue in acetic acid (~60 g/l) TS.

Specific optical rotation. Use a 20 mg/ml solution; $[\alpha]_D^{20\,°C} = +280$ to $+310°$.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 5.0–7.5.

Light-absorbing impurities. Using a freshly prepared 1.8 mg/ml solution in water, measure the absorbances of a 1-cm layer at 280 nm and at 325 nm; the absorbance at each of these wavelengths does not exceed 0.10.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{17}N_2NaO_4S$ in the substance being tested by comparison with benzylpenicillin sodium RS similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.62 ± 0.03.

Additional requirements for Benzylpenicillin sodium for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

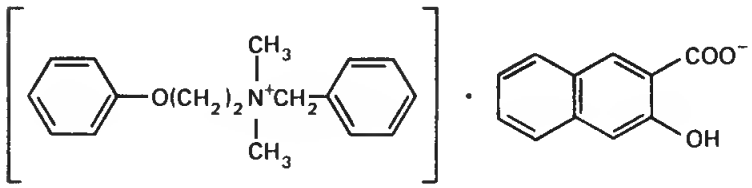
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BEPHENII HYDROXYNAPHTHOAS
BEPHENIUM HYDROXYNAPHTHOATE

Molecular formula. $C_{28}H_{29}NO_4$

Relative molecular mass. 443.5

Graphic formula.



Chemical name. Benzyldimethyl(2-phenoxyethyl)ammonium 3-hydroxy-2-naphthoate (1:1); *N*,*N*-dimethyl-*N*-(2-phenoxyethyl)benzenemethanaminium salt with 3-hydroxy-2-naphthalenecarboxylic acid (1:1); CAS Reg. No. 3818-50-6.

Description. A yellow to greenish yellow, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water, ether R and benzene R; soluble in 50 parts of ethanol (~750 g/l) TS.

Category. Anthelmintic.

Storage. Bephenium hydroxynaphthoate should be kept in a tightly closed container.

Additional information. Even in the absence of light. Bephenium hydroxynaphthoate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Bephenium hydroxynaphthoate contains not less than 99.0% and not more than 101.0%, of $C_{28}H_{29}NO_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from bephenium hydroxynaphthoate RS or with the *reference spectrum* of bephenium hydroxynaphthoate.

B. See the test described below under "Related substances". The principal spots obtained with solution B at 254 nm correspond in position, appearance, and intensity with those obtained with solution C.

C. Melting temperature, about 170 °C with decomposition.

Chlorides. For the preparation of the test solution, boil 0.7 g with 30 ml of water, cool in ice and filter. Add 10 ml of nitric acid (~130 g/l) TS to the filtrate, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.35 mg/g.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 5 volumes of 1-butanol R, 4 volumes of water and 1 volume of acetic acid (~300 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 40 mg of the test substance per ml, (B) 0.40 mg of the test substance per ml, and (C) 0.40 mg of bephenium hydroxynaphthoate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm and 365 nm). At 254 nm two principal spots are visible with each of the solutions A, B, and C, whereas at 365 nm only the spots closer to the solvent front fluoresce. Any additional spot visible with solution A other than the two principal spots, is not more intense in appearance in both lights than the spot obtained closer to the starting line of solution B.

Afterwards, spray the plate first with sodium molybdotungstophosphate TS, then with sodium carbonate (200 g/l) TS, and examine the chromatogram in daylight. Two principal spots are obtained with each of the solutions A, B, and C. Any additional spot obtained with solution A, disregarding those that may have been visible in ultraviolet light, is not more intense than the principal spot closer to the solvent front obtained with solution B.

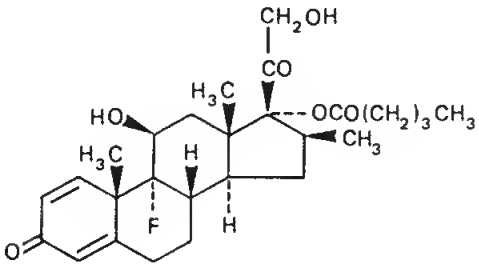
Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 44.35 mg of $C_{28}H_{29}NO_4$.

BETAMETHASONI VALERAS
BETAMETHASONE VALERATE

Molecular formula. $C_{27}H_{37}FO_6$

Relative molecular mass. 476.6

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-valerate; 9-fluoro-11β,21-dihydroxy-16β-methyl-17-[(1-oxopentyl) oxyl]pregna-1,4-diene-3,20-dione; CAS Reg. No. 2152-44-5.

Description. A white or creamy white powder; odourless.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l)TS; freely soluble in acetone R.

Category. Antiinflammatory drug.

Storage. Betamethasone valerate should be kept in a tightly closed container, protected from light.

Requirements

Definition. Betamethasone valerate contains not less than 96.0% and not more than 104.0% of $C_{27}H_{37}FO_6$, calculated with reference to the dried substance.

Identity tests

- Either tests A and B or tests C, D and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from betamethasone valerate RS or with the *reference spectrum* of betamethasone valerate.

B. Dissolve 20 mg in 20 ml of ethanol (~750 g/l) TS and dilute 2 ml to 20 ml with the same solvent. To 2 ml of this solution placed in a stoppered test-tube add 10 ml of phenylhydrazine/sulfuric acid TS, mix, heat in a water-bath at 60 °C for 20 minutes, and cool immediately. The absorbance of a 1-cm layer at the maximum at about 423 nm is not more than 0.25.

C. See the test described below under "Related steroids". The principal spots obtained with solutions A and C correspond in position with that obtained with solution B. In addition, the principal spot obtained with solution A corresponds in appearance and intensity with that obtained with solution B.

D. Carry out the combustion as described under 2.4 Oxygen flask method, using 7 mg of the test substance and a mixture of 0.5 ml of sodium hydroxide (0.01 mol/l) VS and 20 ml of water as the absorbing liquid. When the process is complete, add 0.1 ml of a mixture of 0.1 ml of a freshly prepared sodium alizarinsulfonate (1 g/l) TS and 0.1 ml of zirconyl nitrate TS; the red colour of the solution changes to clear yellow.

E. Heat 0.05 g with 2.0 ml of potassium hydroxide/ethanol TS1 in a water-bath for 5 minutes. Cool, add 2.0 ml of sulfuric acid (~100 g/l) TS, and boil gently for 1 minute; a pleasant odour of ethyl valerate is perceptible.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +75$ to +81 °.

Sulfated ash. Weigh 0.1 g and ignite on a platinum dish; not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related steroids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R, and 0.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 15 mg of betamethasone valerate RS per ml; also apply to the plate 2 µl of a third solution (C) composed of a mixture of equal volumes of solutions A and B and 1 µl of a fourth solution (D) containing 0.15 mg of the test substance per ml in the same solvent mixture used for solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Then heat it at 105 °C for 10 minutes, allow it to cool, spray it with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D.

Assay

- The solutions must be protected from light throughout the assay.

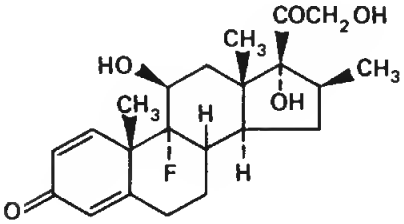
Dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Dilute 20 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS, and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. Calculate the amount of $C_{27}H_{37}FO_6$ in the substance being tested by comparison with betamethasone valerate RS, similarly and concurrently examined.

BETAMETHASONUM
BETAMETHASONE

Molecular formula. $C_{22}H_{29}FO_5$

Relative molecular mass. 392.5

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione; CAS Reg. No. 378-44-9.

Description. A white or creamy white powder; odourless.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Adrenoglucocorticoid.

Storage. Betamethasone should be kept in a tightly closed container, protected from light.

Requirements

Definition. Betamethasone contains not less than 96.0% and not more than 104.0% of $C_{22}H_{29}FO_5$ calculated with reference to the dried substance.

Identity tests

- Either tests A, B and C, or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from betamethasone RS or with the *reference spectrum* of betamethasone (recrystallization from chloroform R of the test substance and the reference substance might be necessary to obtain the same crystalline form).

B. Dissolve 20 mg in 20 ml of ethanol (~750 g/l) TS and dilute 2 ml to 20 ml with the same solvent. To 2 ml of this solution placed in a stoppered test-tube add 10 ml of phenylhydrazine/sulfuric acid TS, mix, heat in a water-bath at 60 °C for 20 minutes, and cool immediately. The absorbance of a 1-cm layer at the maximum at about 450 nm is not more than 0.30 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

C. See the test described below under "Related steroids". The principal spots obtained with solutions A and C correspond in position with that obtained with solution B. In addition the appearance and intensity of the principal spot obtained with solution A corresponds with that obtained with solution B.

D. Carry out the combustion as described under 2.4 Oxygen flask method, using 7 mg of the test substance and a mixture of 0.5 ml of sodium hydroxide (0.01 mol/l) VS and 20 ml of water as the absorbing liquid. When the process is complete, add 0.1 ml to a mixture of 0.1 ml of a freshly prepared sodium alizarinsulfonate (1 g/l) TS and 0.1 ml of zirconyl nitrate TS; the red colour of the solution changes to clear yellow.

Specific optical rotation. Use a 5.0 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +114$ to +122 °.

Sulfated ash. Weigh 0.1 g and use a platinum dish; not more than 5.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related steroids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 15 mg of betamethasone RS per ml; also apply to the plate 2 µl of a third solution (C) composed of a mixture of equal volumes of solutions A and B and 1 µl of a fourth solution (D) containing 0.15 mg of the test substance per ml in the same solvent mixture used for solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated and heat at 105 °C for 10 minutes, allow to cool, spray with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D.

Assay

- The solutions must be protected from light throughout the assay.

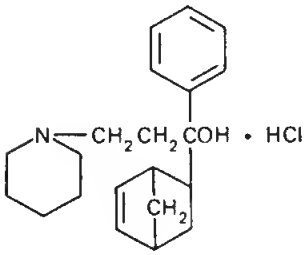
Dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Dilute 20 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l)TS to produce 100 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS, and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) in a similar manner. Calculate the amount of $C_{22}H_{29}FO_5$ in the substance being tested by comparison with betamethasone RS, similarly and concurrently examined.

BIPERIDENI HYDROCHLORIDUM
BIPERIDEN HYDROCHLORIDE

Molecular formula. $C_{21}H_{29}NO,HCl$

Relative molecular mass. 347.9

Graphic formula.



Chemical name. α-5-Norbornen-2-yl-α-phenyl-1-piperidinepropanol hydrochloride; α-bicyclo[2.2.1]hept-5-en-2-yl-α-phenyl-1-piperidinepropanol hydrochloride; CAS Reg. No. 1235-82-1.

Description. A white, crystalline powder; odourless.

Solubility. Slightly soluble in water, ethanol (~750 g/l) TS and ether R; sparingly soluble in methanol R.

Category. Antiparkinsonism drug.

Storage. Biperiden hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Biperiden hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{21}H_{29}NO,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from biperiden hydrochloride RS or with the *reference spectrum* of biperiden hydrochloride.

B. Dissolve 20 mg in 5 ml of phosphoric acid (~1440 g/l) TS and allow to stand; a green colour is produced.

C. Dissolve 0.10 g in 50 ml of water and to 5 ml of this solution add bromine TS1 drop by drop; a yellow precipitate is formed which dissolves on shaking. Upon the addition of more bromine TS1, a permanent precipitate is produced.

D. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 3 hours at 105 °C; it loses not more than 5 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate coated with a suspension of silica gel R1 in sodium hydroxide (0.5 mol/l) VS, and as the mobile phase a mixture of 96.5 volumes of toluene R and 3.5 volumes of methanol R. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 20 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

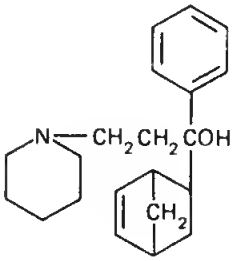
Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, warming slightly to effect solution, add 10 ml of mercuric acetate/acetic acid TS and 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator. Titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 34.79 mg of $C_{21}H_{29}NO,HCl$.

BIPERIDENUM
BIPERIDEN

Molecular formula. $C_{21}H_{29}NO$

Relative molecular mass. 311.5

Graphic formula.



Chemical name. α-5-Norbornen-2-yl-α-phenyl-1-piperidinepropanol; α-bicyclo[2.2.1]hept-5-en-2-yl-α-phenyl-1-piperidinepropanol; CAS Reg. No. 514-65-8.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in ether R; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antiparkinsonism drug.

Storage. Biperiden should be kept in a well-closed container, protected from light.

Requirements

Definition. Biperiden contains not less than 98.0% and not more than 101.0% of $C_{21}H_{29}NO$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from biperiden RS or with the *reference spectrum* of biperiden.

B. Dissolve 20 mg in 5 ml of phosphoric acid (~1440 g/l) TS and allow to stand; a green colour is produced.

C. To 0.20 g add 80 ml of water, 0.5 ml of hydrochloric acid (~70 g/l) TS, and warm until dissolved. Cool and to 5 ml of this solution add bromine TS1 drop by drop; a yellow precipitate is formed which dissolves on shaking. Upon the addition of more bromine TS1, a permanent precipitate is produced.

D. Melting temperature, about 114 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 3 hours at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate coated with a suspension of silica gel R1 in sodium hydroxide (0.5 mol/l) VS, and as the mobile phase a mixture of 96.5 volumes of toluene R and 3.5 volumes of methanol R. Apply separately to the plate 5 µl of each of 2 solutions in chloroform R containing (A) 40 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 31.15 mg of $C_{21}H_{29}NO$.

Additional requirement for Biperiden for parenteral use

Complies with the monograph for "Parenteral preparations".

BLEOMYCINI HYDROCHLORIDUM

BLEOMYCIN HYDROCHLORIDE

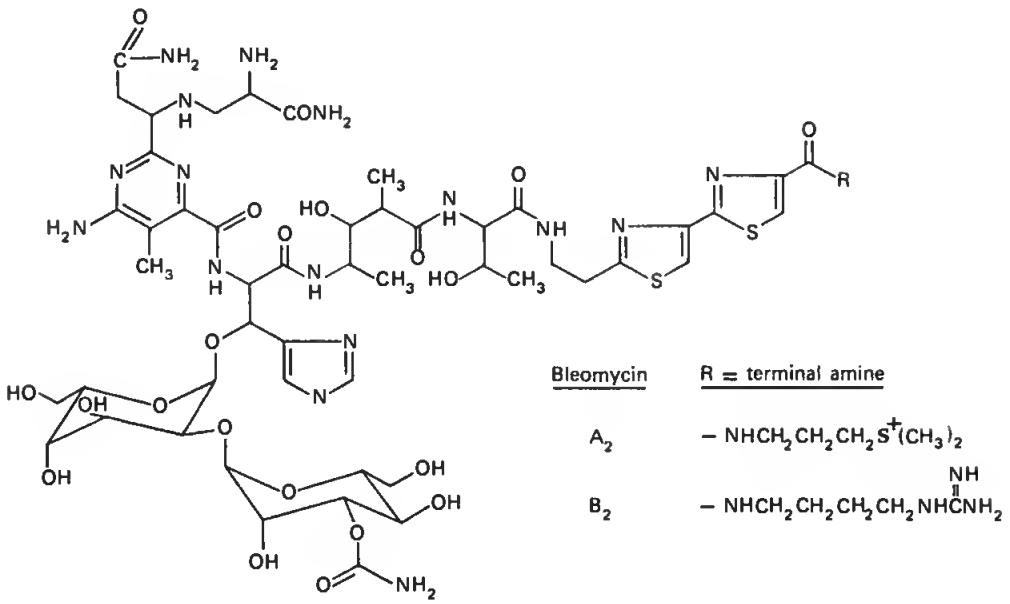
Bleomycin hydrochloride (non-injectable)
Bleomycin hydrochloride, sterile

Chemical name. Bleomycin hydrochloride; CAS Reg. No. 67763-87-5.

Molecular formula. Bleomycin A_2 hydrochloride: $C_{55}H_{84}N_{17}O_{21}S_3,Cl$. Bleomycin B_2 hydrochloride: $C_{55}H_{84}N_{20}O_{21}S_2,HCl$.

Relative molecular mass. Bleomycin A_2 hydrochloride: 1452; Bleomycin B_2 hydrochloride: 1461.

Graphic formulas for the bleomycin A_2/B_2 bases.



Chemical names for the bleomycin A_2/B_2 bases. Bleomycin A_2 hydrochloride: N^1-[3-(Dimethylsulfonio)-propyl]bleomycinamide chloride; [3-[2′-[2-[(2*S*,3*R*)-2-[(2*S*,3*S*,4*R*)-4-[(2*S*,3*R*)-2-[6-amino-2-[(1*S*)-1-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-2-carbamoylethyl]-5-methyl-4-pyrimidinecarboxamido]-3-[[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-3-imidazol-4-ylpropionamido]-3-hydroxy-2-methylvaleramido]-3-hydroxybutyramido]ethyl][2,4′-bithiazole]-4-carboxamido]propyl]dimethylsulfonium chloride; CAS Reg. No. 49830-49-1.

Bleomycin B_2 hydrochloride: N^1-(Guanidinobutyl)bleomycinamide hydrochloride; (β*S*)-4-amino-β-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-6-[[(1*S*,2*R*)-2-[[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-1-[[(1*R*,2*S*,3*S*)-3-[[(1*S*,2*R*)-1-[[2-[4-[(4-guanidinobutyl)carbamoyl][2,4′-bithiazol]-2′-yl]ethyl]carbamoyl]-2-hydroxypropyl]carbamoyl]-2-hydroxy-1-methylbutyl]carbamoyl]-2-imidazol-4-ylethyl]carbamoyl]-5-methyl-2-pyrimidinepropionamide hydrochloride; N^1-[4-[(aminoiminomethyl)amino]butyl]bleomycinamide hydro-chloride; CAS Reg. No. 55658-44-1.

Description. A white to yellowish white powder.

Solubility. Freely soluble in water and in methanol R; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Cytotoxic drug.

Storage. Bleomycin hydrochloride should be kept in a tightly closed container.

Labelling. The designation sterile Bleomycin hydrochloride indicates that the substance complies with the additional requirements for sterile Bleomycin hydrochloride and may be used for parenteral administration or for other sterile applications. CAUTION: Bleomycin hydrochloride must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Bleomycin hydrochloride is the hydrochloride salt of a mixture of substances produced by the growth of *Streptomyces verticillus*. The main components of the mixture are bleomycin A_2 and bleomycin B_2.

Bleomycin hydrochloride contains, when tested according to assay A, not less than 1500 and not more than 2000 International Units of bleomycin A_2/B_2 per mg, calculated with reference to the dried substance.

Further, Bleomycin hydrochloride contains, when tested according to assay B, not less than 55.0% and not more than 70.0% of bleomycin A_2 and not less than 25.0% and not more than 32.0% of bleomycin B_2; the total of bleomycin A_2 and bleomycin B_2 is not less than 85%. The content of bleomycin A_5 is not more than 7.0%, of bleomycin B_4 not more than 1.0%, and of demethylbleomycin A_2 not more than 3.0%.

Identity tests

A. Dissolve about 5 mg in 10 ml of water, add 5 µl of copper(II) sulfate (160 g/l) TS and dilute with water to 100 ml; the absorption spectrum exhibits maxima at about 242 nm and 290 nm, and a minimum at about 268 nm.

B. A 10 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 60 mg/g.

pH value. pH of a 5.0 mg/ml solution, 4.5–6.0.

Copper content. Transfer 75 mg, accurately weighed, to a 60-ml separating funnel and dissolve in 10 ml of hydrochloric acid (0.1 mol/l) VS. Transfer 10 ml of copper standard TS2 to an additional separating funnel. To both funnels add 10 ml of zinc bis(dibenzyldithiocarbamate) TS and shake vigorously for 1 minute. Allow the layers to separate. Filter the lower layer through 1 g of anhydrous sodium sulfate R to remove excess water. Measure the absorbances of a

1-cm layer at the maximum at about 435 nm, using a solvent cell containing carbon tetrachloride R.

Calculate the content of copper in mg/g from the formula $(A_0 \times 15)/(A_s \times W)$ where A_0 is the absorbance of the substance to be examined, A_s is the absorbance of copper standard TS2, and W is the weight in mg of the substance to be examined; the copper content is not more than 0.2 mg/g.

Assay

A. ***Microbiological assay.*** Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Mycobacterium smegmatis* (ATCC 607) as the test organism. Prepare the inoculum as follows: the test organism is grown for 40-48 hours at a temperature of 27 °C on the surface of culture medium Cm8. Using 3 ml of saline TS wash the growth into a flask containing 100 ml of culture medium Cm9 and 50 g of glass beads, and incubate at 25–27 °C for 5 days with constant mechanical agitation using an orbital shaker. The resulting suspension should be used for no longer than 14 days, and kept at a temperature below 5 °C. For the preparation of inoculated plates use 0.5 ml of the suspension or a suitable volume previously determined using test plates with culture medium Cm8 at a temperature of 27 °C. Prepare the reference solution in phosphate buffer, pH 7.0, TS, diluting the International Reference Preparation of bleomycin A_2/B_2 to an appropriate concentration (usually between 10 and 200 µg per ml). The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 1500 IU and the lower fiducial limit is not more than 2000 IU of bleomycin A_2/B_2 per mg, calculated with reference to the dried substance.

B. ***Content of the bleomycin components.*** Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column 25 cm long and 4.6 mm in internal diameter packed with particles of silica gel, 5-10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups. As the mobile phase for a linear gradient development, start with a mixture of 9 volumes of 1-pentanesulfonic acid TS and 1 volume of methanol R, both previously filtered and deaerated, and end with a composition of 6 volumes of 1-pentanesulfonic acid TS and 4 volumes of methanol R, using a suitable linear rate of change of mobile phase so as to reach the final composition in 60 minutes. (If needed, add the following to the mobile phase to obtain satisfactory chromatography: 1.86 g of disodium edetate R per litre.) As detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm, fitted with a low-volume flow cell (8–20 µl is suitable). Inject 5 µl of a solution of the test substance in water containing the equivalent of 5 IU of bleomycin per ml. Proceed

with the gradient elution, pumping the mobile phase mixture at the condition mentioned above for about 80 minutes or until the demethylbleomycin A_2 is eluted.

The elution order of the bleomycin components is the following: void volume, bleomycin acid, bleomycin A_2, bleomycin B_2, bleomycin A_5, bleomycin B_4, and demethylbleomycin A_2.

Calculate in % the content of each bleomycin component, comparing the ratios of the individual areas of the peaks with that of the total area of all the bleomycins.

Additional requirements for Bleomycin hydrochloride for sterile use

Storage. Sterile Bleomycin hydrochloride should be kept in a hermetically closed container.

Manufacture. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine-like substances. Carry out the test as described under 3.6 Test for histamine-like substances (vasodepressor substances) using 1 ml per kg of body mass of a solution in saline TS containing a quantity equivalent to 500 IU per ml.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 10.0 IU of endotoxin RS per mg of bleomycin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BLEOMYCINI SULFAS

BLEOMYCIN SULFATE

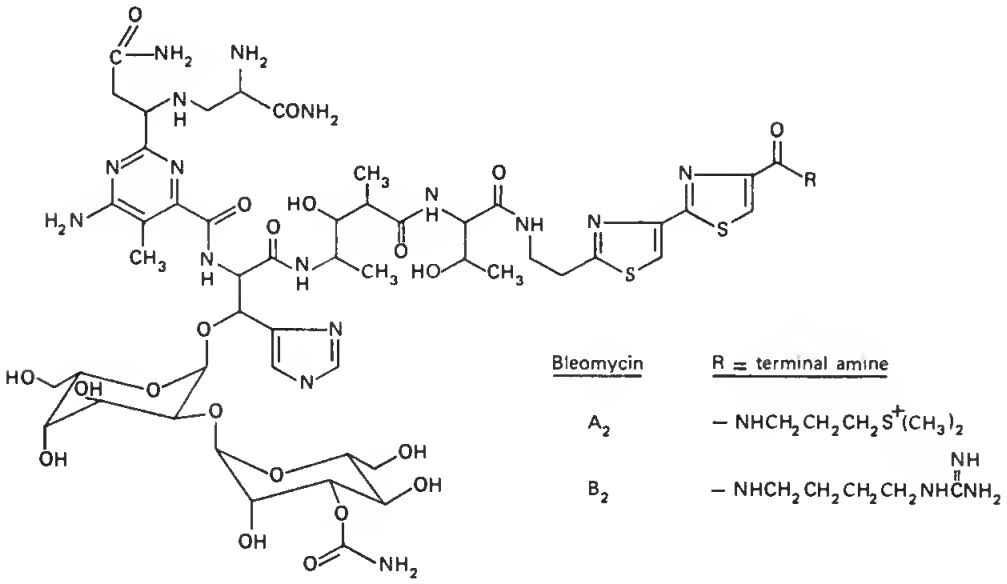
Bleomycin sulfate (non-injectable)
Bleomycin sulfate, sterile

Chemical name. Bleomycin sulfate; CAS Reg. No. 9041-93-4.

Molecular formula. Bleomycin A_2 sulfate: $C_{55}H_{84}N_{17}O_{21}S_3,H_2SO_4$; Bleomycin B_2 sulfate: $C_{55}H_{84}N_{20}O_{21}S_2,H_2SO_4$

Relative molecular mass. Bleomycin A_2 sulfate: 1514; Bleomycin B_2 sulfate: 1524.

Graphic formulas for the bleomycin A_2/B_2 bases.



Chemical names for the bleomycin A_2/B_2 bases. Bleomycin A_2 sulfate: N^1-[3-(Dimethylsulfonio)propyl]bleomycinamide hydrogen sulfate; [3-[2′-[2-[(2*S*,3*R*)-2-[(2*S*,3*S*,4*R*)-4-[(2*S*,3*R*)-2-[6-amino-2-[(1*S*)-1-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-2-carbamoylethyl]-5-methyl-4-pyrimidinecarboxamido]-3-[[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-3-imidazol-4-ylpropionamido]-3-hydroxy-2-methylvaleramido]-3-hydroxybutyramido]ethyl][2,4′-bithiazole]-4-carboxamido]propyl]dimethylsulfonium hydrogen sulfate.

Bleomycin B_2 sulfate: N^1-(Guanidinobutyl)bleomycinamide; (β*S*)-4-amino-β-[[(2*S*)-2-amino-2-carbamoylethyl]amino]-6-[[(1*S*,2*R*)-2-[[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]oxy]-1-[[(1*R*,2*S*,3*S*)-3-[[(1*S*,2*R*)-1-[[2-[4-[(4-guanidinobutyl)carbamoyl][2,4′-bithiazol]-2′-yl]ethyl]carbamoyl]-2-hydroxypropyl]carbamoyl]-2-hydroxy-1-methylbutyl]carbamoyl]-2-imidazol-4-ylethyl]carbamoyl]-5-methyl-2-pyrimidinepropionamide sulfate (salt); N^1-[4-[(aminoiminomethyl)amino]butyl]bleomycinamide sulfate (salt).

Description. A white or cream-coloured, amorphous powder.

Solubility. Very soluble in water.

Category. Cytotoxic drug.

Storage. Bleomycin sulfate should be kept in a tightly closed container.

Labelling. The designation sterile Bleomycin sulfate indicates that the substance complies with the additional requirements for sterile Bleomycin sulfate and may be used for parenteral administration or for other sterile applications. CAUTION: Bleomycin sulfate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Bleomycin sulfate is the sulfate salt of a mixture of substances produced by the growth of *Streptomyces verticillus*. The main components of the mixture are bleomycin A_2 and bleomycin B_2.

Bleomycin sulfate contains, when tested according to assay A, not less than 1500 and not more than 2000 International Units of bleomycin A_2/B_2 per mg, calculated with reference to the dried substance.

Further, Bleomycin sulfate contains, when tested according to assay B, not less than 55.0% and not more than 70.0% of bleomycin A_2 and not less than 25.0% and not more than 32.0% of bleomycin B_2; the total of bleomycin A_2 and bleomycin B_2 is not less than 85%. The content of bleomycin A_5 is not more than 7.0%, of bleomycin B_4 not more than 1.0%, and of demethylbleomycin A_2 not more than 3.0%.

Identity tests

A. Dissolve about 5 mg in 10 ml of water, add 5 µl of copper(II) sulfate (160 g/l) TS, and dilute with water to 100 ml; the absorption spectrum exhibits maxima at about 242 nm and 290 nm, and a minimum at about 268 nm.

B. A 10 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 60 mg/g.

pH value. pH of a 5.0 mg/ml solution, 4.5–6.0.

Copper content. Transfer 75 mg, accurately weighed, to a 60-ml separating funnel and dissolve in 10 ml of hydrochloric acid (0.1 mol/l) VS. Transfer 10 ml of copper standard TS2 to an additional separating funnel. To both funnels add 10 ml of zinc bis(dibenzyldithiocarbamate) TS and shake vigorously for 1 minute. Allow the layers to separate. Filter the lower layer through 1 g of anhydrous sodium sulfate R to remove excess water. Measure the absorbances of a

1-cm layer at the maximum at about 435 nm, using a solvent cell containing carbon tetrachloride R.

Calculate the content of copper in mg/g from the formula $(A_0 \times 15)/(A_s \times W)$ where A_0 is the absorbance of the substance to be examined, A_s is the absorbance of copper standard TS2, and W is the weight in mg of the substance to be examined; the copper content is not more than 0.2 mg/g.

Assay

A. ***Microbiological assay.*** Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Mycobacterium smegmatis* (ATCC 607) as the test organism. Prepare the inoculum as follows: the test organism is grown for 40–48 hours at a temperature of 27 °C on the surface of culture medium Cm8. Using 3 ml of saline TS wash the growth into a flask containing 100 ml of culture medium Cm9 and 50 g of glass beads, and incubate at 25–27 °C for 5 days with constant mechanical agitation using an orbital shaker. The resulting suspension should be used for no longer than 14 days, and kept at a temperature below 5 °C. For the preparation of inoculated plates use 0.5 ml of the suspension or a suitable volume previously determined using test plates with culture medium Cm8 at a temperature of 27 °C. Prepare the reference solution in phosphate buffer, pH 7.0, TS, diluting the International Reference Preparation of bleomycin A_2/B_2 to an appropriate concentration (usually between 10 and 200 µg per ml). The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 1500 IU and the lower fiducial limit is not more than 2000 IU of bleomycin A_2/B_2 per mg, calculated with reference to the dried substance.

B. ***Content of the bleomycin components.*** Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column 25 cm long and 4.6 mm in internal diameter packed with particles of silica gel, 5–10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups. As the mobile phase for a linear gradient development, start with a mixture of 9 volumes of 1-pentanesulfonic acid TS and 1 volume of methanol R, both previously filtered and deaerated, and end with a composition of 6 volumes of 1-pentanesulfonic acid TS and 4 volumes of methanol R, using a suitable linear rate of change of mobile phase so as to reach the final composition in 60 minutes. (If needed, add the following to the mobile phase to obtain satisfactory chromatography: 1.86 g of disodium edetate R per litre.) As detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm, fitted with a low-volume flow cell (8–20 µl is suitable). Inject 5 µl of a solution of the test substance in water containing the equivalent of 5 IU of bleomycin per ml. Proceed

with the gradient elution, pumping the mobile phase mixture at the condition mentioned above for about 80 minutes or until the demethylbleomycin A_2 is eluted.

The elution order of the bleomycin components is the following: void volume, bleomycin acid, bleomycin A_2, bleomycin B_2, bleomycin A_5, bleomycin B_4, and demethylbleomycin A_2.

Calculate in % the content of each bleomycin component, comparing the ratios of the individual areas of the peaks with that of the total area of all the bleomycins.

Additional requirements for Bleomycin sulfate for sterile use

Storage. Sterile Bleomycin sulfate should be kept in a hermetically closed container.

Manufacture. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine-like substances. Carry out the test as described under 3.6 Test for histamine-like substances (vasodepressor substances) using 1 ml per kg of body mass of a solution in saline TS containing a quantity equivalent to 500 IU per ml.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 10.0 IU of endotoxin RS per mg of bleomycin.

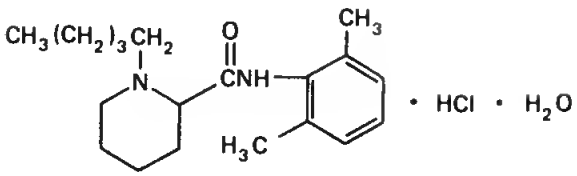
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

BUPIVACAINI HYDROCHLORIDUM
BUPIVACAINE HYDROCHLORIDE

Molecular formula. $C_{18}H_{28}N_2O,HCl,H_2O$

Relative molecular mass. 342.9

Graphic formula.



Chemical name. 1-Butyl-2′,6′-pipecoloxylidide monohydrochloride monohydrate; 1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide monohydrochloride monohydrate; CAS Reg. No. 73360-54-0.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 25 parts of water and in 8 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Local anaesthetic.

Storage. Bupivacaine hydrochloride should be kept in a well-closed container.

Requirements

Definition. Bupivacaine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{18}H_{28}N_2O$,HCl, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from bupivacaine hydrochloride RS or with the *reference spectrum* of bupivacaine hydrochloride.

B. Dissolve 0.15 g in 10 ml of water and add 20 ml of trinitrophenol (7 g/l) TS. Heat the mixture to boiling, allow to cool and, if necessary, scrape the inner surface of the beaker to induce crystallization; wash the precipitate rapidly with a small quantity of water, followed by successive quantities of methanol R and ether R, using 2 ml each time; melting temperature about 194 °C (bupivacaine picrate).

C. A 2 mg/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of chlorides.

Copper. To 0.25 g in 10 ml of water, add 0.25 ml of disodium edetate (0.05 mol/l) VS, and allow to stand for 2 minutes; add 0.2 g of copper-free citric

acid R, 1 ml of ammonia (~100 g/l) TS and 1 ml of sodium diethyldithiocarbamate (0.8 g/l) TS and extract with 10 ml of carbon tetrachloride R for 2 minutes. The colour of the extract is not deeper than that of the extract obtained when 10 ml of a mixture of 3 volumes of copper (II) sulfate (80 g/l) TS and 397 volumes of water are similarly treated.

Iron. Ignite 1.0 g with 1 g of anhydrous sodium carbonate FeR; cool and dissolve the residue in 5 ml of hydrochloric acid (~250 g/l) FeTS and 30 ml of water. Treat the solution as described under 2.2.4 Limit test for iron, using 0.5 ml of iron standard FeTS; the iron content is not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 45 mg/g and not more than 60 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5–6.0.

Absorption in the ultraviolet region. The absorption spectrum of a 0.4 mg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 263 nm and 271 nm. The absorbance of a 1-cm layer at the maximum wavelength of 263 nm is not less than 0.53 and not more than 0.58, and at the maximum wavelength of 271 nm is not less than 0.43 and not more than 0.48 (preferably use 2-cm cells for the measurements and calculate the absorbances of 1-cm layers).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and ethanol (~750 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.50 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.65 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 32.49 mg of $C_{18}H_{28}N_2O,HCl$.

Additional requirements for Bupivacaine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

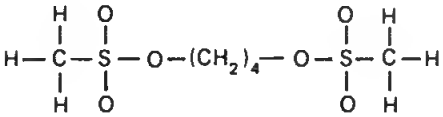
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.5 IU of endotoxin RS per mg.

BUSULFANUM
BUSULFAN

Molecular formula. $C_6H_{14}O_6S_2$

Relative molecular mass. 246.3

Graphic formula.



Chemical name. 1,4-Butanediol dimethanesulfonate; tetramethylene dimethanesulfonate; CAS Reg. No. 55-98-1.

Other name. Myelosanum.

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water; sparingly soluble in acetone R; slightly soluble in ethanol (~750 g/l) TS.

Category. Cytotoxic drug.

Storage. Busulfan should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Busulfan must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Busulfan contains not less than 98.5% and not more than 100.5% of $C_6H_{14}O_6S_2$, calculated with reference to the dried substance.

Identity tests

A. Heat 0.1 g with 10 ml of water and 5 ml of sodium hydroxide (1 mol/l) VS until a clear solution is obtained; an intense odour of methanesulfonic acid is perceptible. Cool the solution and divide it into two equal portions for test B.

B. To one portion of the solution prepared in test A add 0.05 ml of potassium permanganate (10 g/l) TS; the purple colour changes to violet, then to blue, and finally to emerald-green. Acidify the second portion of the solution prepared in test A with 2 ml of sulfuric acid (~100 g/l) TS, add 0.05 ml of potassium permanganate (10 g/l) TS and shake; the colour of the permanganate is slowly discharged.

C. To a test-tube transfer 0.10 g of the test substance, suspend it in 1.0 ml of copper edetate TS and 0.5 ml of ammonia (~260 g/l) TS, then add 0.5 ml of hydrogen peroxide (~60 g/l) TS; this constitutes solution 1. Similarly, prepare a blank without the test substance; this constitutes solution 2. Place both tubes in a water-bath for 5 minutes, cool and add 1.0 ml of hydrochloric acid (~70 g/l) TS and 4.0 ml of barium chloride (50 g/l) TS; solution 2 remains clear and an opalescence is produced in solution 1, which changes to a white precipitate after a few minutes.

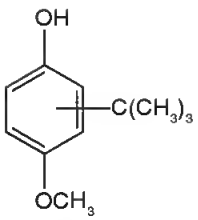
Melting range. 115–118 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 20 mg/g.

Assay. To about 0.25 g, accurately weighed, add 25 ml of water and boil gently under reflux for 30 minutes. Wash the condenser with a small quantity of water, cool, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 12.32 mg of $C_6H_{14}O_6S_2$.

BUTYLHYDROXYANISOLUM
BUTYLATED HYDROXYANISOLE



$C_{11}H_{16}O_2$

Relative molecular mass. 180.3

Chemical name. *tert*-Butyl-4-methoxyphenol; (1,1-dimethylethyl)-4-methoxyphenol; CAS Reg. No. 25013-16-5.

Other name. BHA.

Description. A white or almost white, crystalline powder or a yellowish white solid; odour, faint and characteristic.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS, ether R, propylene glycol R, and arachis oil R; dissolves in solutions of alkali hydroxides.

Category. Antioxidant.

Storage. Butylated hydroxyanisole should be kept in a well-closed container, protected from light.

Requirements

Definition. Butylated hydroxyanisole contains a variable amount of 3-*tert*-butyl-4-methoxyphenol.

Identity tests

A. Dissolve 0.1 g in 10 ml of ethanol (~750 g/l) TS and add 4 ml of sodium tetraborate (10 g/l) TS and 1 ml of 2,6-dichloroquinone chlorimide/ethanol TS; a blue colour is produced (distinction from butylated hydroxytoluene).

B. Dissolve a few crystals in 10 ml of ethanol (~750 g/l) TS and add 0.1 ml of potassium ferricyanide (10 g/l) TS and 0.5 ml of ferric ammonium sulfate TS2; a green to blue colour is produced.

Solution in methanol. A solution of 1 g in 10 ml of methanol R is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Hydroquinone. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 4 volumes of chloroform R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 3 ml of each of two solutions in ether R containing (A) 50 mg of Butylated hydroxyanisole per ml, and (B) 0.10 mg of hydroquinone R per ml. After removing the plate from the chromatographic chamber,

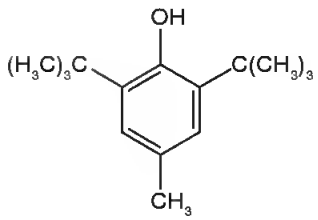
allow it to dry in air for a few minutes, spray with phosphomolybdic acid/ethanol TS, and while still damp expose it to the vapours of ammonia (~260 g/l) TS. Examine the chromatogram in daylight as soon as the yellow background has disappeared.

The spot obtained with solution B is more intense than any corresponding spot obtained with solution A.

3-*tert*-Butyl-4-methoxyphenol. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of three solutions in ether R containing (A) 25 mg of Butylated hydroxyanisole per ml, (B) 2.5 mg of Butylated hydroxyanisole per ml, and (C) 0.125 mg of Butylated hydroxyanisole per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with ferric chloride/potassium ferricyanide TS, and examine the chromatogram in daylight.

The blue-violet spot at R_f ~35 obtained with solution A is not more intense than the principal spot obtained with solution B. Any other spot obtained with solution A is not more intense than the spot obtained with solution C.

BUTYLHYDROXYTOLUENUM
BUTYLATED HYDROXYTOLUENE



$C_{15}H_{24}O$

Relative molecular mass. 220.4

Chemical name. 2,6-Di-*tert*-butyl-*p*-cresol; 2,6-bis(1,1-dimethylethyl)-4-methyl-phenol; CAS Reg. No. 128-37-0.

Other name. BHT.

Description. Colourless crystals or a white or almost white, crystalline powder; odour, faint and characteristic.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS, acetone R, ether R, and arachis oil R.

Category. Antioxidant.

Storage. Butylated hydroxytoluene should be kept in a well-closed container, protected from light.

Requirements

Identity tests

A. Dissolve 0.1 g in 10 ml of ethanol (~750 g/l) TS and add 4 ml of sodium tetraborate (10 g/l) TS and a few crystals of 2,6-dichloroquinone chlorimide R; no more than a faint blue colour is produced (distinction from butylated hydroxyanisole).

B. Dissolve 10 mg in 2 ml of ethanol (~750 g/l) TS. Add 1 ml of testosterone propionate/ethanol TS and 2 ml of sodium hydroxide (~80 g/l) TS. Warm in a water-bath at 80 °C for 10 minutes, and allow to cool; a blue colour is produced.

Congealing temperature. Not lower than 69.2 °C.

Sulfated ash. Not more than 1.0 mg/g.

Acid value. Not more than 0.05.

CALAMINUM

CALAMINE

Chemical name. Calamine; CAS Reg. No. 8011-96-9.

Description. A fine, amorphous pink or reddish brown powder; odourless.

Solubility. Practically insoluble in water; soluble with effervescence in mineral acids.

Category. Antipruritic drug.

Storage. Calamine should be kept in a well-closed container.

Additional information. Attention should be paid to the microbiological quality since Calamine is of natural origin.

Requirements

Definition. Calamine is zinc oxide with a small proportion of ferric oxide.

Calamine contains not less than **98.0%** and not more than the equivalent of **100.5%** of ZnO, calculated with reference to the ignited substance.

Identity tests

A. Shake 1 g with 10 ml of hydrochloric acid (~70 g/l) TS and filter. To 5 ml of the filtrate add 0.3 ml of sodium hydroxide (~80 g/l) TS; a white precipitate is formed. Add a further 2 ml of sodium hydroxide (~80 g/l) TS; the precipitate dissolves. Add 10 ml of ammonium chloride (100 g/l) TS; the solution remains clear. Add 0.1 ml of sodium sulfide TS; a white, flocculent precipitate is formed.

B. To 1 g add 10 ml of hydrochloric acid (~70 g/l) TS, heat to boiling, and filter. To the filtrate add a few drops of ammonium thiocyanate (75 g/l) TS; a reddish colour is produced.

Calcium or magnesium. Digest 1 g in 25 ml of hydrochloric acid (~70 g/l) TS for 30 minutes and filter. To the filtrate, add slowly ammonia (~100 g/l) TS until the precipitate first formed redissolves, then add an excess of 5 ml of ammonia (~100 g/l) TS. To 10 ml of this solution add 2 ml of ammonium oxalate (25 g/l) TS; not more than a slight turbidity is produced. To a further 10 ml portion add 2 ml of disodium hydrogen phosphate (100 g/l) TS; not more than a slight turbidity is produced.

Lead. Dissolve 2 g in a mixture of 20 ml of water and 5 ml of glacial acetic acid R, filter, and add 0.1 ml of potassium chromate (100 g/l) TS to the filtrate; the solution remains clear for 5 minutes.

Acid-insoluble substances. Dissolve 2.0 g in 50 ml of hydrochloric acid (~70 g/l) TS, and filter. Wash the residue with water and dry to constant mass at 105 °C; the residue weighs not more than 40 mg (2.0%).

Alkaline substances. Digest 1 g with 20 ml of water and warm on a water-bath for 15 minutes. Filter and add 2 drops of phenolphthalein/ethanol TS to the filtrate; if a red colour is produced, titrate with sulfuric acid (0.05 mol/l) VS; not more than 0.2 ml of acid is required to discharge the colour.

Ethanol-soluble dyes. Shake 1 g with 10 ml of ethanol (~710 g/l) TS and filter; the filtrate is colourless.

Water-soluble dyes. Shake 1 g with 10 ml of water and filter; the filtrate is colourless.

Loss on ignition. Weigh 2.0 g and ignite at 500 °C to constant mass; it loses not more than 20 mg/g.

Assay. Add to about 1.5 g, accurately weighed, 50 ml of sulfuric acid (0.5 mol/l) VS, heat gently until no further precipitation occurs, and filter. Wash the residue with hot water until the last washing is neutral to litmus paper R. Combine the wash liquid and the filtrate, add 2.5 g of ammonium chloride R, cool, and back-titrate with sodium hydroxide (1 mol/l) VS using methyl orange/ethanol TS as indicator.

Each ml of sulfuric acid (0.5 mol/l) VS is equivalent to 40.69 mg of ZnO.

CALCII CARBONAS
CALCIUM CARBONATE

Molecular formula. $CaCO_3$

Relative molecular mass. 100.1

Chemical name. Calcium carbonate (1:1); CAS Reg. No. 471-34-1.

Description. A white, fine, microcrystalline powder; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS. It dissolves with effervescence in acetic acid (~60 g/l) TS, hydrochloric acid (~70 g/l) TS, and nitric acid (~130 g/l) TS.

Category. Antacid.

Storage. Calcium carbonate should be kept in a well-closed container.

Requirements

Definition. Calcium carbonate contains not less than 98.0% and not more than 100.5% of $CaCO_3$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 20 mg in 0.3 ml of hydrochloric acid (~70 g/l) TS and 2 ml of water, and filter. The filtrate yields the reactions described under 2.1 General identification tests as characteristic of calcium.

B. To 0.10 g add 1.0 ml of acetic acid (~300 g/l) TS; a gas evolves that is colourless and odourless. Pass the evolved gas into calcium hydroxide TS; a white precipitate is produced immediately.

Heavy metals. Dissolve 5 g in 80 ml of acetic acid (~60 g/l) TS; when effervescence ceases, boil the solution for 2 minutes, allow to cool, dilute to 100 ml with acetic acid (~60 g/l) TS and, if necessary, filter through a sintered glass filter (retain the filter for the test of substances insoluble in acetic acid). Determine the heavy metals content in 20 ml of the filtrate (keep the remaining filtrate for the limit test for barium), as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 30 µg/g.

Arsenic. Use a solution of 3.3 g in 35 ml of hydrochloric acid (~70 g/l) TS and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Barium. To 10 ml of the filtrate retained from the limit test for heavy metals add 10 ml of calcium sulfate TS (solution A). Mix a further 10 ml of the filtrate with 10 ml of water (solution B). After not less than 15 minutes, solution A is not more opalescent than solution B.

Iron. Dissolve 0.20 g in 10 ml of hydrochloric acid (~70 g/l) TS and dilute to 40 ml with water. Proceed with the 2.2.4 Limit test for iron; not more than 200 µg/g.

Magnesium and alkali metals. Dissolve 1.0 g in 10 ml of hydrochloric acid (~70 g/l) TS, boil for 2 minutes and add 20 ml of water, 1 g of ammonium chloride R, and 0.1 ml of methyl red/ethanol TS. Add ammonia (~100 g/l) TS drop by drop until the solution changes colour, and then add a further 2 ml. Heat to boiling and add 40 ml of hot ammonium oxalate (50 g/l) TS. Allow to stand for 4 hours, dilute to 100 ml with water and filter. To 50 ml of the filtrate add 0.25 ml of sulfuric acid (~100 g/l) TS and evaporate to dryness on a water-bath. Ignite the residue to constant weight at 600 °C; not more than 5 mg.

Substances insoluble in acetic acid. Wash the filter retained from the test for heavy metals with 4 successive quantities, each of 5 ml of hot water, and dry at 105 °C for 1 hour; the residue weighs not more than 10 mg.

Loss on drying. Dry to constant weight at 200 °C; it loses not more than 20 mg/g.

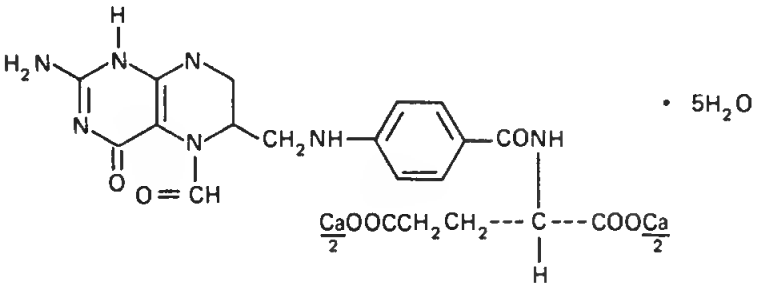
Assay. Dissolve about 0.15 g, accurately weighed, in a mixture of 3 ml of hydrochloric acid (~70 g/l) TS and 20 ml of water, boil for 2 minutes, allow to cool, and dilute to 50 ml with water. Proceed with the titration as described under 2.5 Complexometric titrations for calcium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 5.004 mg of $CaCO_3$.

CALCII FOLINAS
CALCIUM FOLINATE

Molecular formula. $C_{20}H_{21}CaN_7O_7,5H_2O$

Relative molecular mass. 601.6

Graphic formula.



Chemical name. Calcium *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-L-glutamate (1:1) pentahydrate; calcium *N*-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamate (1:1) pentahydrate; CAS Reg. No. 6035-45-6 (pentahydrate).

Other name. Leucovorin calcium.

Description. A white or creamy white powder; odourless.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Cytotoxic drug.

Storage. Calcium folinate should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Calcium folinate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Calcium folinate contains not less than 95.0% and not more than 105.0% of $C_{20}H_{21}CaN_7O_7$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and C or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from calcium folinate RS or with the *reference spectrum* of calcium folinate.

B. Dissolve 20 mg in 3.0 ml of water, add 0.5 ml of hydrochloric acid (~70 g/l) TS and 0.5 ml of sodium nitrite (100 g/l) TS. Shake for 2 minutes and add 1.5 ml of 2-naphthol TS1; a yellow-brown precipitate appears and the solution turns green.

C. Dissolve 20 mg in 2.0 ml of water and add 1.0 ml of ammonium oxalate (25 g/l) TS; a white precipitate is produced, which is insoluble in acetic acid (~300 g/l) TS and ammonia (~260 g/l) TS, but is soluble in hydrochloric acid (~70 g/l) TS.

D. Dissolve 20 mg in 5 ml of water and add 1.0 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Add a few drops of nitric acid (~130 g/l) TS; the precipitate dissolves.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 0.080 g/g and not more than 0.150 g/g.

Assay

- Use freshly deionized water throughout the procedure, and perform the assay in low-actinic glassware or protect the solutions containing calcium folinate from light. Complete the assay without prolonged interruption.

Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column 30 cm long and 4 mm in internal diameter, packed with particles of porous silica gel or ceramic, 5–10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups.

As the mobile phase, use a mixture of 15 ml of tetrabutylammonium hydroxide/methanol TS with 835 ml of water, add 125 ml of acetonitrile R, adjust the pH to 7.5 ± 0.1 with sodium dihydrogen phosphate (275 g/l) TS, dilute with water to 1000 ml, and filter. Adjust the concentration of acetonitrile, if necessary.

Dilute the following solution for use in the preparation of the test solutions: To 15 ml of tetrabutylammonium hydroxide/methanol TS add 900 ml of water,

adjust the pH to 7.5 ± 0.1 with sodium dihydrogen phosphate (275 g/l) TS, dilute with water to 1000 ml, and mix. Weigh accurately a quantity of calcium folinate RS, dissolve it in the above solution and dilute with the same solution to contain about 175 µg per ml (solution A). Dissolve 20 mg of the substance to be examined in a sufficient volume of the above solution to produce 100 ml, and mix (solution B). For the system suitability test, dissolve a quantity of folic acid RS in the above solution and dilute with the same solution to contain about 175 µg per ml. Mix 1 part of this solution with 4 parts of solution A (solution C).

Operate at a flow rate of 1–2 ml per minute. As detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm, fitted with a suitable recorder.

Make 6 replicate injections, each of 15 µl of solution C. The resolution factor between calcium folinate and folic acid should be not less than 3.6, with a relative standard deviation for the calcium folinate peak of not more than 2.0%. The relative retention times for calcium folinate and folic acid are 1.0 and about 1.6, respectively.

Then inject 15 µl of each of solutions A and B. Measure the peak responses at the corresponding retention times and calculate the quantity, in %, of $C_{20}H_{21}CaN_7O_7$, using the following formula: $100(0.1C)(r_U/r_S)$ in which C is the concentration in µg per ml of calcium folinate RS in solution A, and r_U and r_S are the peak responses obtained from solutions B and A, respectively.

Additional requirements for Calcium folinate for parenteral use

Complies with the monograph for "Parenteral preparations".

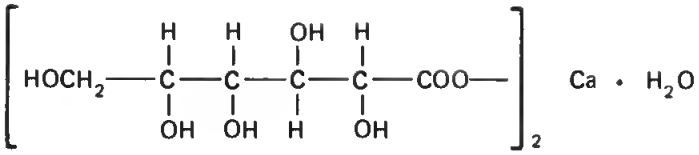
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.

CALCII GLUCONAS
CALCIUM GLUCONATE

Molecular formula. $(C_6H_{11}O_7)_2Ca,H_2O$

Relative molecular mass. 448.4

Graphic formula.



Chemical name. Calcium D-gluconate (1:2) monohydrate; CAS Reg. No. 299-28-5.

Description. White, crystalline granules or a white, crystalline powder; odourless.

Solubility. Slowly soluble in water; freely soluble in boiling water; practically insoluble in dehydrated ethanol R and ether R.

Category. Calcium source.

Storage. Calcium gluconate should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Calcium gluconate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Calcium gluconate contains not less than 98.0% and not more than 102.0% of $(C_6H_{11}O_7)_2Ca,H_2O$, calculated as the monohydrate.

Identity tests

A. A 20 mg/ml solution yields the reactions described under 2.1 General identification tests as characteristic of calcium.

B. To 1 ml of a 30 mg/ml solution add 1 drop of ferric chloride (25 g/l) TS; a yellow colour is produced.

C. To 5 ml of a warm 0.1 g/ml solution add 0.7 ml of glacial acetic acid R and 1 ml of freshly distilled phenylhydrazine R, heat on a water-bath for 30 minutes, allow to cool, and scrape the inner surface of the tube to induce crystallization. Collect the crystals, dissolve in 10 ml of hot water, add a small amount of charcoal R, and filter. Allow the filtrate to cool, and scrape the inner surface of the tube; a white, crystalline precipitate is produced; melting temperature, about 200 °C with decomposition (phenylhydrazide of gluconic acid).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides and other halides. Dissolve 0.50 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.5 mg/g.

Magnesium and alkaline metals. Dissolve 1.0 g in 100 ml of boiling water, add 10 ml of ammonium chloride (100 g/l) TS, 1 ml of ammonia (~260 g/l) TS and, drop by drop, 50 ml of hot ammonium oxalate (25 g/l) TS. Allow to stand for 4 hours, dilute to 200 ml with water and filter. Evaporate 100 ml of the filtrate to dryness and ignite; the residue weighs not more than 2.0 mg.

Sulfates. Dissolve 5.0 g in 40 ml of boiling water, cool and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.1 mg/g.

Glucose and sucrose. Dissolve 0.5 g in 10 ml of hot water, add 2 ml of hydrochloric acid (~70 g/l) TS, and boil for about 2 minutes. Cool, add 15 ml of sodium carbonate (50 g/l) TS, allow to stand for 5 minutes, and filter. Add 5 ml of the clear filtrate to about 2 ml of potassio-cupric tartrate TS, and boil for 1 minute; neither a red turbidity nor any precipitate is produced.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

Assay. Dissolve about 0.5 g, accurately weighed, in 20 ml of hot water containing 2 ml of hydrochloric acid (~70 g/l) TS, allow to cool and dilute to 100 ml with water. Proceed with the titration as described under 2.5 Complexometric titrations for calcium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 22.42 mg of $(C_6H_{11}O_7)_2Ca,H_2O$.

Additional requirements for Calcium gluconate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 167 IU of endotoxin RS per g.

CALCII HYDROGENOPHOSPHAS
CALCIUM HYDROGEN PHOSPHATE

Calcium hydrogen phosphate, anhydrous
Calcium hydrogen phosphate dihydrate

$CaHPO_4$ (anhydrous)
$CaHPO_4,2H_2O$ (dihydrate)

Relative molecular mass. 136.1 (anhydrous); 172.1 (dihydrate).

Chemical name. Calcium phosphate (1:1); CAS Reg. No. 7757-93-9 (anhydrous).

Calcium phosphate (1:1) dihydrate; CAS Reg. No. 7789-77-7 (dihydrate).

Other name. Dibasic calcium phosphate.

Description. A white or almost white powder; odourless.

Solubility. Practically insoluble in cold water and ethanol (~750 g/l) TS; soluble in dilute acids.

Category. Tablet and capsule diluent.

Storage. Calcium hydrogen phosphate should be kept in a well-closed container.

Labelling. The designation on the container of Calcium hydrogen phosphate should state whether it is the dihydrate or the anhydrous form.

Requirements

Calcium hydrogen phosphate contains not less than **30.9%** and not more than the equivalent of **31.7%** of calcium, Ca, calculated with reference to the ignited substance.

Identity tests

A. To 0.2 g add a mixture of 10 ml of hydrochloric acid (~70 g/l) TS and 10 ml of water, and heat to dissolve. To 10 ml of this solution add 2.5 ml of ammonia (~100 g/l) TS (keep the remaining solution for test B); it yields reaction A described under 2.1 General identification tests as characteristic of calcium.

B. Acidify the remaining solution from test A with nitric acid (~130 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in 10 ml of hydrochloric acid (~70 g/l) TS, filter if necessary, and add ammonia (~100 g/l) TS until a precipitate is formed. Add just sufficient hydrochloric acid (~70 g/l) TS to dissolve the precipitate and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 40 µg/g.

Arsenic. Use a solution of 1.0 g in 35 ml of hydrochloric acid (~70 g/l) TS and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Barium. Dissolve 1.25 g in 10 ml of hydrochloric acid (~70 g/l) TS, filter if necessary, and add ammonia (~100 g/l) TS until a precipitate is formed. Add just sufficient hydrochloric acid (~70 g/l) TS to dissolve the precipitate and dilute with water to 25 ml. Place a 10-ml portion in each of two separate matched tubes. To one portion add 0.5 ml of sulfuric acid (~100 g/l) TS, and to the other 0.5 ml of water; the solutions remain equally clear when viewed after 15 minutes.

Carbonates. To 1 g add 5 ml of carbon-dioxide-free water R and 2 ml of hydrochloric acid (~420 g/l) TS and shake; no effervescence is produced.

Chlorides. Dissolve 0.1 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 2.5 mg/g.

Fluorides. Prepare and store all solutions in plastic containers.

Weigh 2.0 g of the test sample into a beaker and add 20 ml of water and 2.0 ml of hydrochloric acid (~250 g/l) TS. Using a magnetic stirrer and a plastic-coated stirring bar, stir until the sample has dissolved. Then add 50 ml of sodium citrate (250 g/l) TS and dilute to 100 ml with water. Use a fluoride-ion-sensitive electrode and a silver/silver chloride reference electrode system, connected to a potentiometer capable of indicating reproducibly a minimum of ±0.2 mV. Insert the previously rinsed and dried electrodes into the solution, stir for 5 minutes, and read the potential in mV.

Prepare a standard solution of fluoride ion containing 1.1052 mg sodium fluoride R per ml. To 20 ml of this solution add 50 ml of sodium citrate (250 g/l) TS and dilute with sufficient water to produce 100 ml (100 µg F/ml). For the establishment of a standard curve, place 50 ml of sodium citrate (250 g/l) TS in a beaker, add 2 ml of hydrochloric acid (~250 g/l) TS, and dilute to 100 ml with water. Stir as described above for 15 minutes, insert the electrodes, and read the potential in mV. Continue to stir, and at 5-minute intervals add 100 µl, 100 µl, 300 µl, and 500 µl of fluoride ion standard solution (100 µg F/ml), equivalent to the cumulative fluoride ion concentration of 0.1, 0.2, 0.5, and 1.0 µg/ml,

reading the potential 5 minutes after each addition. Plot the logarithms of the cumulative fluoride ion concentration versus potential.

Determine the concentration of fluoride ion in the solution being examined, reading off from the standard curve the value of mV correlating with the µg of F/ml, and divide by the sample mass taken to obtain the content in the sample; not more than 50 µg/g.

Sulfates. Dissolve 0.10 g in 5 ml of hydrochloric acid (~70 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 5 mg/g.

Acid-insoluble substances. To 5 g add a mixture of 40 ml of water and 10 ml of hydrochloric acid (~420 g/l) TS, heat until no more dissolves, and dilute to 100 ml with water. Filter any residue, wash with hot water until the washing is free of chlorides, dry the residue at 105 °C for 1 hour, and weigh; not more than 2 mg/g.

Loss on ignition. Ignite 1.0 g to constant mass between 800 and 825 °C. The anhydrous form loses not less than 66 mg/g and not more than 85 mg/g. The dihydrate loses not less than 0.245 g/g and not more than 0.265 g/g.

Assay. To about 0.2 g, accurately weighed, add a mixture of 1 ml of hydrochloric acid (~420 g/l) TS and 5 ml of water, use gentle heat to dissolve, and add 125 ml of water. Proceed with the titration as described under 2.5 Complexometric titrations for calcium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.004 mg of Ca.

CALCII PHOSPHAS
CALCIUM PHOSPHATE

Chemical name. Calcium phosphate (3:2) mixture with calcium phosphate (1:1); CAS Reg. No. 7758-87-4 [$Ca_3(PO_4)_2$]; CAS Reg. No. 7757-93-9 ($CaHPO_4$).

Other name. Tribasic calcium phosphate.

Description. A white, amorphous powder; odourless or almost odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in hydrochloric acid (~70 g/l) TS and nitric acid (~130 g/l) TS.

Category. Tablet diluent.

Storage. Calcium phosphate should be kept in a well-closed container.

Additional information. At relative humidities between 15% and 65%, the equilibrium moisture content at 25 °C is about 2%, but at relative humidities above 75% small additional amounts of moisture are absorbed.

Requirements

Definition. Calcium phosphate is a mixture consisting mainly of $Ca_3(PO_4)_2$ together with $CaHPO_4$.

Calcium phosphate contains not less than **34.0%** and not more than the equivalent of **40.0%** of calcium, Ca, calculated with reference to the ignited substance.

Identity tests

A. Dissolve 0.05 g in 1 ml of hydrochloric acid (~70 g/l) TS by gentle warming and add 4 ml of water and 0.5 g of sodium acetate R. It yields reaction A described under 2.1 General identification tests as characteristic of calcium.

B. To 0.5 g add 2 ml of nitric acid (~130 g/l) TS and heat gently. This solution yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in 10 ml of hydrochloric acid (~70 g/l) TS. Heat to boiling, cool, dilute to 40 ml with water, and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 30 µg/g.

Arsenic. Use a solution of 3.3 g in 35 ml of hydrochloric acid (~70 g/l) TS, heat to dissolve and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Barium. Mix 0.5 g with 10 ml of water, heat, and add, drop by drop, hydrochloric acid (~420 g/l) TS until solution is effected. Add an excess of 2 drops of acid, filter, and to the filtrate add 1 ml of potassium sulfate (0.1 g/l) TS; no turbidity appears within 15 minutes.

Carbonates. Suspend 5 g in 30 ml of carbon-dioxide-free water R and add slowly 10 ml of hydrochloric acid (~70 g/l) TS; not more than a slight effervescence is observed. (Keep the solution for "Acid-insoluble substances".)

Chlorides. Dissolve 0.2 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 1.4 mg/g.

Fluorides. Prepare and store all solutions in plastic containers.

Weigh 2.0 g of the test sample into a beaker, add 20 ml of water and 3 ml of hydrochloric acid (~250 g/l) TS. Using a magnetic stirrer and a plastic-coated stirring bar, stir until the sample has dissolved. Then add 50 ml of sodium citrate (250 g/l) TS and dilute to 100 ml with water. Use a fluoride-ion-sensitive electrode and a silver/silver chloride reference electrode system, connected to a potentiometer capable of indicating reproducibly a minimum of ±0.2 mV. Insert the previously rinsed and dried electrodes into the solution, stir for 5 minutes, and read the potential in mV.

Prepare a standard solution of fluoride ion containing 1.1052 mg sodium fluoride R per ml. To 20 ml of this solution add 50 ml of sodium citrate (250 g/l) TS and dilute with sufficient water to produce 100 ml (100 µg F/ml). For the establishment of a standard curve, place 50 ml of sodium citrate (250 g/l) TS in a beaker, add 3 ml of hydrochloric acid (~250 g/l) TS, and dilute to 100 ml with water. Stir as described above for 15 minutes, insert the electrodes, and read the potential in mV. Continue to stir, and at 5-minute intervals add 100 µl, 100 µl, 300 µl, 500 µl, and 500 µl of fluoride ion standard solution (100 µg F/ml), equivalent to the cumulative fluoride ion concentration of 0.1, 0.2, 0.5, 1.0, and 1.5 µg/ml, reading the potential 5 minutes after each addition. Plot the logarithms of the cumulative fluoride ion concentration versus potential.

Determine the concentration of fluoride ion in the solution being examined, reading off from the standard curve the value of mV correlating with the µg of F/ml, and divide by the sample mass taken to obtain the content in the sample; not more than 75 µg/g.

Sulfates. Dissolve 0.1 g in 5 ml of hydrochloric acid (~70 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 8 mg/g.

Acid-insoluble substances. Filter the solution prepared for "Carbonates", wash the residue with water, and dry to constant mass at 105 °C; the residue weighs not more than 15 mg (0.3%).

Loss on ignition. Ignite 1.0 g at 800 °C for 30 minutes; it loses not more than 80 mg/g.

Assay. To about 0.15 g, accurately weighed, add a mixture of 5 ml of hydrochloric acid (~420 g/l) TS and 3 ml of water, use gentle heat to dissolve, and add 125 ml of water. Proceed with the titration as described under 2.5 Complexometric titrations for calcium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.004 mg of Ca.

CALCII STEARAS
CALCIUM STEARATE

$(H_3C—[CH_2]_{16}—CO_2)_2Ca$

$C_{36}H_{70}CaO_4$

Chemical name. Calcium stearate; calcium octadecanoate; CAS Reg. No. 1592-23-0.

Description. A white to yellowish white, fine, bulky powder; odour, slight, characteristic.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, acetone R, and ether R.

Category. Tablet and capsule lubricant.

Storage. Calcium stearate should be kept in a well-closed container.

Additional information. The degree of lubrication depends on the particular form and size of the material.

Requirements

Definition. Calcium stearate consists of calcium salts mainly of stearic acid and palmitic acid in variable proportions.

Calcium stearate contains not less than **9.0%** and not more than the equivalent of **10.5%** of CaO, calculated with reference to the dried substance.

Identity tests

A. Heat 1 g with a mixture of 25 ml of water and 5 ml of hydrochloric acid (~420 g/l) TS; fatty acids are liberated and float as an oil on the surface of the liquid. The aqueous layer yields the reactions described under 2.1 General identification tests as characteristic of calcium.

B. Mix 25 g with 200 ml of hot water, add 60 ml of sulfuric acid (~100 g/l) TS, and heat the mixture until the separated fatty acids layer is clear. Wash it with boiling water until free from sulfates, transfer it to a beaker, and warm on a water-bath until the water separates and the fatty acids are clear. Allow to cool, pour off the water layer, melt the fatty acids, and filter into a dry beaker. Dry at 105 °C for 20 minutes; congealing temperature, not lower than 54 °C.

Loss on drying. Heat at 105 °C for 2 hours and weigh; repeat the heating using 2-hour increments until a constant mass is obtained; not more than 40 mg/g.

Assay. To about 1.2 g, accurately weighed, add 50 ml of hydrochloric acid (0.1 mol/l) V, and heat to boiling for 10 minutes or until the separated fatty acids layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and the flask thoroughly with water until the washing is free from acid when tested with litmus paper R. Neutralize the filtrate with sodium hydroxide (1 mol/l) VS against litmus paper R and proceed with the titration as described under 2.5 Complexometric titrations for calcium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.804 mg of CaO.

CALCII SULFAS
CALCIUM SULFATE

$CaSO_4,2H_2O$

Relative molecular mass. 172.2

Chemical name. Calcium sulfate (1 : 1) dihydrate; CAS Reg. No. 10101-41-4.

Description. A white to almost white, fine powder; odourless or almost odourless.

Solubility. Slightly soluble in water; more soluble in dilute mineral acids; practically insoluble in most organic solvents.

Category. Tablet and capsule diluent.

Storage. Calcium sulfate should be kept in a well-closed container.

Requirements

Calcium sulfate contains not less than **98.0%** and not more than the equivalent of **101.0%** of $CaSO_4$, calculated with reference to the dried substance.

Identity tests

Dissolve 1 g in 20 ml of a solution prepared by mixing equal volumes of water and hydrochloric acid (~420 g/l) TS. Heat to boiling for 2 minutes, cool, and filter if necessary. Use this solution for the following tests:

A. The solution yields the reactions described under 2.1 General identification tests as characteristic of calcium.

B. The solution yields the reactions described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. To 1.0 g add 10 ml of water and 20 ml of hydrochloric acid (~70 g/l) TS, heat to boiling until dissolved, cool, and adjust the pH as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity of solution. Dissolve 1 g in a mixture of 45 ml of water and 5 ml of hydrochloric acid (~420 g/l) TS, heating to 50 °C for 5 minutes; the solution is clear.

Loss on drying. Dry to constant mass at a temperature not lower than 250 °C; it loses not less than 190 mg/g and not more than 230 mg/g.

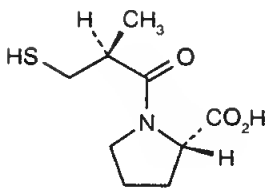
pH value. Slurry 20 g with 80 ml of carbon-dioxide-free water R, allow to settle, and filter, 6.0–7.6.

Assay. To about 0.3 g, accurately weighed, add a mixture of 100 ml of water and 6 ml of hydrochloric acid (~70 g/l) TS, heat to boiling until dissolved, and allow to cool. Proceed with the titration as described under 2.5 Complexometric titrations for calcium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 6.807 mg of $CaSO_4$.

CAPTOPRILUM

CAPTOPRIL



$C_9H_{15}NO_3S$

Relative molecular mass. 217.3

Chemical name. 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-L-proline; 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline; CAS Reg. No. 62571-86-2.

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water, dichloromethane R, and methanol R.

Category. Cardiovascular agent; angiotensin-converting enzyme inhibitor.

Storage. Captopril should be kept in a tightly closed container, protected from light.

Additional information. Captopril may exist in different polymorphic forms.

Requirements

Captopril contains not less than **98.0%** and not more than **102.0%** of $C_9H_{15}NO_3S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from captopril RS or with the *reference spectrum* of captopril.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 75 volumes of toluene R, 25 volumes of glacial acetic acid R, and 1 volume of methanol R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in dichloromethane R containing (A) 5.0 mg of Captopril per ml, and (B) 5.0 mg of captopril RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with 5,5¢- dithiobis-2-nitrobenzoic acid/methanol TS. Examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 25 mg in 2 ml of ethanol (~750 g/l) TS, add a few crystals of sodium nitrite R and 10 ml of sulfuric acid (~100 g/l) TS, and shake; a red colour is produced.

D. Melting temperature, about 107 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -125°$ to -134 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (12.5 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). Prepare the following solution to be used as the mobile phase: mix 0.05 volumes of phosphoric acid (~1440 g/l) TS with 50 volumes of methanol R and 50 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 0.5 mg of Captopril per ml; solution (B) 10 µg of Captopril per ml; and for solution (C) dissolve 10 µg of Captopril in the mobile phase, add 1 ml of iodine (0.05 mol/l) VS, and dilute to 100 ml with the mobile phase; further dilute 10 ml of this solution to 100 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 220 nm.

Inject 20 µl of solution B and adjust the sensitivity of the system so that the height of the principal peak is not less than 40% of the full scale of the recorder. Inject 20 µl of solution C. The test is not valid unless three peaks are obtained and the resolution between the last two eluting principal peaks is at least 2.0.

Inject alternately 20 µl each of solutions A and B. Continue the chromatography for three times the retention time of the principal peak obtained with solution A.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak obtained with solution B (1.0%). The sum of the areas of all the peaks, other than the principal peak, is not greater than the area of the peak obtained with solution A (2.0%). Disregard any peak with a retention time of less than 1.4 minutes or with an area less than 0.1 times that of the peak obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 100 ml of water, add 10 ml of sulfuric acid (~190 g/l) TS and 1 g of potassium iodide R. Mix and titrate with

potassium iodate (0.01 mol/l) VS, using starch TS as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium iodate required.

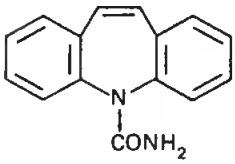
Each ml of potassium iodate (0.01 mol/l) VS is equivalent to 13.04 mg of $C_9H_{15}NO_3S$.

CARBAMAZEPINUM
CARBAMAZEPINE

Molecular formula. $C_{15}H_{12}N_2O$

Relative molecular mass. 236.3

Graphic formula.



Chemical name. 5*H*-Dibenz[*b,f*]azepine-5-carboxamide; CAS Reg. No. 298-46-4.

Description. A white to yellowish white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water and ether R; soluble in ethanol (~750 g/l) TS.

Category. Antiepileptic drug.

Storage. Carbamazepine should be kept in a tightly closed container.

Requirements

Definition. Carbamazepine contains not less than 98.0% and not more than 102.0% of $C_{15}H_{12}N_2O$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum obtained from the test substance without pretreatment is concordant with the spectrum obtained from carbamazepine RS or with the *reference spectrum* of carbamazepine.

B. See the test described below under "Related substances". The principal spot obtained with solution C corresponds in position, appearance, and intensity with that obtained with solution D.

C. Expose a small amount of the test substance to ultraviolet light (365 nm); an intense blue fluorescence is observed.

D. Heat 0.1 g with 2 ml of nitric acid (~1000 g/l) TS in a water-bath for 3 minutes; an orange-red colour is produced.

Melting range. 189–193 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity or alkalinity. Stir 1.0 g with 20 ml of carbon-dioxide-free water R for 15 minutes and filter. To 10 ml of the filtrate add 0.1 ml of phenolphthalein/ethanol TS and titrate with carbonate-free sodium hydroxide (0.01 mol/l) VS; not more than 0.5 ml is required to obtain a pink colour. Add 0.15 ml of methyl red/ethanol TS and titrate with hydrochloric acid (0.01 mol/l) VS; not more than 1.0 ml is required to obtain a red colour.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 86 volumes of toluene R and 14 volumes of methanol R as the mobile phase. Apply separately to the plate 2 µl of each of 5 solutions in a mixture of equal volumes of ethanol (~750 g/l) TS and chloroform R containing (A) 0.050 g of the test substance per ml, (B) 0.050 mg of iminodibenzyl R per ml, (C) 5.0 mg of the test substance per ml, (D) 5.0 mg of carbamazepine RS per ml, and (E) 5.0 µg of carbamazepine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with potassium dichromate TS3, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Then heat the plate at 140 °C for 15 minutes and examine the

chromatogram in ultraviolet light (254 nm). Any additional spot obtained with solution A is not more intense than that obtained with solution E.

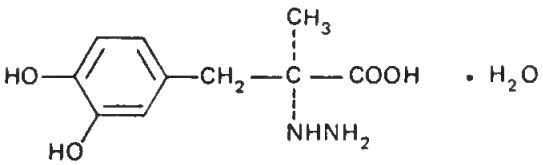
Assay. Dissolve about 0.1 g, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml. Dilute 10 ml of this solution to 100 ml with the same solvent, and again dilute 10 ml of this dilution to 100 ml with ethanol (~750 g/l) TS. Measure the absorbance of a 1-cm layer of the resulting solution at the maximum at about 285 nm. Calculate the amount of $C_{15}H_{12}N_2O$ in the substance being tested by comparison with carbamazepine RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.49 ± 0.02.

CARBIDOPUM
CARBIDOPA

Molecular formula. $C_{10}H_{14}N_2O_4,H_2O$

Relative molecular mass. 244.2

Graphic formula.



Chemical name. (-)-L-α-Hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate; (*S*)-α-hydrazino-3,4-dihydroxy-α-methylbenzenepropanoic acid monohydrate; CAS Reg. No. 38821-49-7 (monohydrate).

Description. A white to creamy white powder; odourless or almost odourless.

Solubility. Slightly soluble in water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiparkinsonism drug.

Storage. Carbidopa should be kept in a well-closed container, protected from light.

Requirements

Definition. Carbidopa contains not less than 99.0% and not more than 101.0% of $C_{10}H_{14}N_2O_4$, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from carbidopa RS or with the *reference spectrum* of carbidopa.

B. To 5 mg add 1 ml of water, 1 ml of pyridine R, and 5 mg of 4-nitrobenzoyl chloride R, mix and allow to stand for 3 minutes; the solution remains colourless, but after boiling changes to a pale yellow colour. While shaking, add 0.1 ml of sodium carbonate (200 g/l) TS; an orange colour is produced.

Specific optical rotation. Use a 10 mg/ml solution in aluminium chloride TS and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = -22.5$ to $-26.5\,°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not less than 69 mg/g and not more than 79 mg/g.

Methyldopa and 3-*O*-Methylcarbidopa. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column 20 cm long and 4 mm in internal diameter packed with particles of silica gel, 10 µm in diameter, the surface of which has been modified with chemically bonded octylsilyl groups. As the mobile phase, use a mixture of 98 volumes of potassium dihydrogen phosphate (13.6 g/l) TS and 2 volumes of methanol R at a flow rate of 1.5 ml per minute. As detector use an ultraviolet spectrophotometer at a wavelength of about 282 nm, fitted with a low-volume flow cell (10 µl is suitable).

Prepare the following solutions in hydrochloric acid (0.1 mol/l) VS containing (A) 0.050 mg of methyldopa RS, 0.050 mg of (-)-3-(4-hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine RS and 0.10 mg of (-)-3-(4-hydroxy-3-methoxyphenyl)-2-methylalanine RS per ml, the last serving as an internal standard, (B) 10 mg of the test substance per ml, and (C) 10 mg of the test substance and 0.10 mg of the internal standard per ml.

In the chromatogram obtained with solution A the peaks, excluding the solvent peak, are due to (*a*) methyldopa, (*b*) (-)-3-(4-hydroxy-3-methoxyphenyl)-2-methylalanine and (*c*) (-)-3-(4-hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine in order of their emergence. The ratios of the areas of the peaks (*a*) and (*c*) to the area of the peak due to the internal standard are greater than the corresponding ratios in the chromatogram obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in 25.0 ml of perchloric acid (0.1 mol/l) VS with the aid of a minimum of heat. Titrate the excess perchloric acid with sodium acetate/glacial acetic acid (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 22.62 mg of $C_{10}H_{14}N_2O_4$.

CARBO ACTIVATUS
CHARCOAL, ACTIVATED

Description. Fine, black powder, free from grittiness; odourless.

Solubility. Practically insoluble in water and in all usual solvents.

Category. General-purpose antidote; pharmaceutic aid.

Storage. Activated Charcoal should be kept in a well-closed container.

Additional information. Activated Charcoal is a tasteless powder.

Requirements

Identity test. Heat a small quantity of the test substance to redness; it burns slowly without a flame.

Heavy metals. Boil 1 g with a mixture of 20 ml of hydrochloric acid (~70 g/l) TS and 5 ml of bromine TS1 for 5 minutes, filter, and wash with 50 ml of boiling water. Evaporate the combined filtrates to dryness on a water-bath and add to the residue 1 ml of hydrochloric acid (1 mol/l) VS, 20 ml of water, and 5 ml of sulfurous acid TS. Boil the solution until all the sulfur dioxide has been expelled, filter if necessary, and dilute with water to 50 ml. Use 10 ml as the test solution and determine the content of heavy metals as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 100 µg/g.

Cyanides. In a distillation apparatus, heat 5 g carefully with 50 ml of water and 2 g of tartaric acid R. Collect about 25 ml of distillate in a mixture of 10 ml

of water and 2 ml of sodium hydroxide (1 mol/l) VS and dilute to 50 ml with water. To 25 ml add 0.05 g of ferrous sulfate R and heat until boiling starts. Cool in a water-bath at 70 °C and acidify with 10 ml of hydrochloric acid (~250 g/l) TS; no green or blue colour develops.

Sulfides. To 1 g in a small conical flask, add 20 ml of water and 5 ml of hydrochloric acid (~250 g/l) TS; the escaping vapours do not darken a strip of filter paper moistened with lead acetate (80 g/l) TS.

Zinc. To 1 g add 25 ml of nitric acid (~130 g/l) TS and heat to boiling for 5 minutes; filter through sintered glass and wash with 10 ml of hot water. Determine the content of zinc either by a dithizone method (A) or by atomic absorption spectrophotometry (B):

A. To 10 ml of the clear solution obtained as described above add successively 3.0 ml of water, 3.0 ml of sodium acetate (60 g/l) TS, 5.0 ml of cyanide/oxalate/thiosulfate TS, and 5.0 ml of a freshly prepared 30 mg/ml solution of dithizone R in carbon tetrachloride R, Mix thoroughly for 2–3 minutes. Separate the dithizone-layer and place in a suitable comparison tube. To 0.5 ml of zinc standard (20 µg/ml Zn) TS add 9.5 ml of water and treat it in the same manner as above. The solution of the test substance shows by reflection a more intense violet colour and, by transmitted light, a not more intense violet colour than the reference solution.

B. Dilute appropriately the solution obtained as described above and proceed as described under 1.8 Atomic spectrometry: emission and absorption.

Fluorescent substances. In an apparatus for intermittent extraction, treat 10 g with 100 ml of cyclohexane R1 for 2 hours. Collect the cyclohexane extract, adjust the volume to 100 ml, and examine in ultraviolet light (365 nm). The fluorescence of the solution is not more intense than that of a solution containing 0.083 mg of quinine R in 1000 ml of sulfuric acid (0.005 mol/l) VS.

Ethanol-soluble substances. In a flask fitted with a reflux condenser, heat 2 g with 50 ml of ethanol (~750 g/l) TS. Boil for 10 minutes, filter immediately, cool, and readjust the volume to 50 ml with ethanol (~750 g/l) TS; the filtrate is not more intensely coloured than reference solution Yw1. Evaporate 40 ml of the filtrate, dry the residue at 105 °C, and weigh; not more than 8 mg (5 mg/g).

Acid-soluble substances. Boil 1 g with a mixture of 20 ml of water and 5 ml of hydrochloric acid (~420 g/l) TS for 5 minutes, filter into a tared porcelain crucible, and wash the residue with 10 ml of hot water, adding the washings to the filtrate. To the combined filtrate and washings add 1 ml of sulfuric acid (~1760 g/l) TS, evaporate to dryness, and ignite to constant weight; not more than 35 mg/g.

Alkali-soluble coloured matter. Heat 0.25 g with 10 ml of sodium hydroxide (~80 g/l) TS for 1 minute, cool and filter. Dilute the filtrate to 10 ml with water; the colour is not more intense than reference solution Gn2.

Sulfated ash. Not more than 50 mg/g.

Loss on drying. Dry for 4 hours at 120 °C; it loses not more than 150 mg/g.

Acidity or alkalinity. To 2 g add 40 ml of water and heat to boiling for 5 minutes. Cool, restore to the original volume with freshly boiled and cooled water and filter. Reject the first 20 ml of filtrate. The filtrate does not induce any colour change in red or blue litmus paper R. To 10 ml of the filtrate add 0.25 ml of bromothymol blue/ethanol TS and 0.25 ml of sodium hydroxide (0.02 mol/l) VS; the solution is blue. Add 0.75 ml of hydrochloric acid (0.02 mol/l) VS; the solution turns yellow.

Adsorbing power

A. Place 1 g, previously dried at 120 °C for 4 hours, in a solution of 100 mg of strychnine sulfate R in 50 ml of water and shake for 5 minutes; filter, rejecting the first 10 ml of filtrate. To a 10-ml portion of the filtrate add 1 drop of hydrochloric acid (~420 g/l) TS and 5 drops of potassio-mercuric iodide TS; no turbidity is produced.

B. To each of two glass-stoppered 100-ml flasks transfer 50 ml of methylthioninium chloride (1 g/l) TS. To one of the flasks add 0.25 g, accurately weighed, of the test substance, insert the stopper in the flask and shake for 5 minutes. Filter the contents of each flask, rejecting the first 20 ml of each filtrate. Transfer 25-ml portions of the filtrates to two 250-ml volumetric flasks. Add to each flask 50 ml of sodium acetate (60 g/l) TS, mix, and add from a burette 35.0 ml of iodine (0.05 mol/l) VS, swirling the mixture during the addition. Stopper the flasks and allow them to stand for 50 minutes, shaking them vigorously at 10-minute intervals. Dilute each mixture with water to volume, mix, allow to stand for 10 minutes, and filter, rejecting the first 30 ml of each filtrate. Titrate the excess iodine in a 100-ml aliquot of each filtrate with sodium thiosulfate (0.1 mol/l) VS, adding 3 ml of starch TS towards the end of the titration. Calculate the number of ml of iodine (0.05 mol/l) VS consumed in each titration; the difference between the two volumes is not less than 0.7 ml.

CARBOMERUM
CARBOMER

Chemical name. Acrylic acid polymer with sucrose polyalkyl ether; carbomer; CAS Reg. No. 9007-20-9.

Description. A white, fluffy powder; odour, slight, characteristic.

Solubility. After neutralization with alkali hydroxides or amines, soluble in water, ethanol (~750 g/l) TS, and glycerol R.

Category. Suspending agent.

Storage. Carbomer should be kept in a tightly closed container.

Additional information. Carbomer is very hygroscopic.

Requirements

Definition. Carbomer is a synthetic high molecular mass polymer of allyl acid copolymerized with polysucrose.

Carbomer contains not less than **56.0%** and not more than the equivalent of **68.0%** of carboxylic acid groups (—COOH), calculated with reference to the dried substance.

Identity tests

A. Disperse 0.5 g in 50 ml of water. To 10 ml add a few drops of thymol blue/ethanol TS; the colour of the dispersion is orange. To a further 10 ml add a few drops of cresol red/ethanol TS; the colour is yellow. (Keep the dispersion for test B.)

B. Adjust the pH of the dispersion from test A to about 7.5 with sodium hydroxide (1 mol/l) VS; a very viscous gel is produced.

Yield value. Prepare a gel as follows: Carefully add 2.5 g to 500 ml of water containing 0.25 g of sodium chloride R in a 1000-ml beaker, stirring continuously at 990–1010 revolutions per minute, the stirrer shaft set to one side of the beaker and near to the bottom at an angle of 60° from the vertical. Add Carbomer being examined slowly at a uniform rate over 45–90 seconds, ensuring that any loose aggregates of powder are broken up. Continue to stir for 15 minutes, remove the stirrer, and allow the beaker containing the dispersion to stand in a water-bath at a temperature of 24.8–25.2 °C for 30 minutes. Insert the stirrer to a depth such that air is not drawn into the dispersion and, while

stirring at 290–310 revolutions per minute, add 0.2 ml of phenolphthalein/ethanol TS and 1.5 ml of bromothymol blue/ethanol TS. Add rapidly below the surface 5 ml of sodium hydroxide (~200 g/l) TS and stir for 2–3 minutes until neutralization is reached, indicated by a uniform blue colour. Adjust the pH to 7.3–7.8 potentiometrically, using glass and calomel electrodes, either adding more sodium hydroxide (~200 g/l) TS or preparing a new mucilage using less sodium hydroxide for the neutralization. Return the neutralized mucilage to the water-bath maintained at 25 °C for 1 hour.

The apparatus consists of two clear soda-glass plates, 100 mm × 100 mm × 3 mm. Rub together by hand fine carborundum paste using two opposing faces of the plates to obtain an even and matt surface. With a diamond marker engrave the plates to show centre and corner alignments and four sample location points equidistant from the plate centre and the four corners.

Place the plates in a water-bath at 24.8–25.2 °C to settle, and dry them rapidly before use. Apply 0.1 g of the mucilage to the matt surface of one of the plates at each location for the sample. Align the second plate and lower it carefully, matt side downwards, onto the lower plate. Top the apparatus with a weight so that the combined mass applied equals 100 g. Allow the assembly to stand for 10 minutes and measure the diameters of the 4 zones of each sample using a strip of paper calibrated in mm; the mean diameter of the zones does not exceed 2.0–2.2 cm.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 80 °C for 1 hour; it loses not more than 20 mg/g.

Assay. Slowly add 0.4 g, accurately weighed and previously dried at 80 °C for 1 hour, to 400 ml of water while mixing with a magnetic stirrer until completely dissolved. At reduced stirring speed, titrate potentiometrically, using glass and calomel electrodes, with sodium hydroxide (0.2 mol/l) VS. After each addition of sodium hydroxide and before recording the pH of the solution, allow to stir for 1 minute.

Each ml of sodium hydroxide (0.2 mol/l) VS is equivalent to 9.004 mg of carboxylic acid groups (—COOH).

CARMELLOSUM NATRICUM
CARMELLOSE SODIUM

Chemical name. Cellulose carboxymethyl ether, sodium salt; CAS Reg. No. 9004-32-4.

Other name. Carboxymethylcellulose sodium.

Description. A white to faintly yellowish powder or granules; odourless.

Solubility. Easily dispersed in water giving a colloidal solution; practically insoluble in acetone R, ethanol (~750 g/l) TS, ether R, and toluene R.

Category. Suspending agent; tablet binder and disintegrant; viscosity-increasing agent.

Storage. Carmellose sodium should be kept in a tightly closed container.

Labelling. The designation on the container of Carmellose sodium should state its viscosity.

Additional information. Carmellose sodium is hygroscopic after drying. This substance is not necessarily suitable for the manufacture of parenteral preparations.

Requirements

Definition. Carmellose sodium is the sodium salt of a partially substituted polycarboxymethyl ether of cellulose.

Carmellose sodium contains not less than **6.5%** and not more than the equivalent of **10.8%** of Na, calculated with reference to the dried substance.

Identity tests

A. Sprinkle 1.0 g of powdered Carmellose sodium onto 90 ml of carbon-dioxide-free water R at 40–50 °C, stir vigorously until a colloidal solution is produced, cool, and dilute to 100 ml with carbon-dioxide-free water R. Transfer 0.5 ml to a test-tube (keep the remaining solution for "Chlorides", "Clarity and colour of solution", and "pH value"), add 1 ml of water and 5 drops of 1-naphthol TS1, mix, and carefully introduce down the side of the tube 2 ml of sulfuric acid (~1760 g/l) TS to form a lower layer; a red-violet colour develops at the interface.

B. To the sulfated ash, add 1 ml of hydrochloric acid (~420 g/l) TS, evaporate to dryness on a water-bath, and dissolve the residue in 20 ml of water. Use 5 ml, keeping the remaining solution for "Heavy metals"; it yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Heavy metals. Use 12 ml of the solution remaining from identity test B and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 40 µg/g.

Chlorides. Use 10 ml of the solution prepared for identity test A and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 2.5 mg/g.

Clarity and colour of solution. The solution prepared in identity test A is not more opalescent than opalescence standard TS3 and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Use 1.0 g and a mixture of equal volumes of sulfuric acid (~1760 g/l) TS and water. Calculate the result with reference to the dried substance; 0.200 g/g – 0.333 g/g corresponding to a content of Na equivalent to 6.5–10.8%. (Keep the residue for identity test B.)

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 100 mg/g.

pH value. pH of the solution prepared for identity test A, 6.0–8.5.

CELLACEFATUM
CELLACEFATE

Chemical name. Cellulose acetate phthalate; cellulose acetate 1,2-benzenedicarboxylate; CAS Reg. No. 9004-38-0.

Other names. Cellulose acetate phthalate; cellacephate.

Description. A white, free-flowing powder or colourless flakes; odourless or with a faint odour of acetic acid.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; freely soluble in acetone R; soluble in dioxan R; dissolves in dilute solutions of alkali.

Category. Enteric coating agent for solid oral dosage forms.

Storage. Cellacefate should be kept in a well-closed container, and stored in a cool and dry place.

Additional information. Cellacefate is hygroscopic.

Requirements

Definition. Cellacefate is a cellulose, some of the hydroxyl groups of which are esterified by phthaloyl groups and others by acetyl groups.

Cellacefate contains not less than **30.0%** and not more than the equivalent of **40.0%** of phthaloyl groups ($C_8H_5O_3$, relative molecular mass = 149.1) and not less than **17.0%** and not more than the equivalent of **26.0%** of acetyl groups (C_2H_3O, relative molecular mass = 43.05), both calculated with reference to the anhydrous substance.

Identity tests

A. To 10 mg add 1.0 ml of ethanol (~750 g/l) TS and 1 ml of sulfuric acid (~1760 g/l) TS, and warm; ethyl acetate, perceptible by its odour (*proceed with caution*), is produced.

B. Transfer 10 mg to a small test-tube, add 10 mg of resorcinol R and 0.5 ml of sulfuric acid (~1760 g/l) TS, and mix. Heat in a liquid bath at 160 °C for 3 minutes. Cool and pour the solution into a mixture of 25 ml of sodium hydroxide (1 mol/l) VS and 200 ml of water; a vivid green fluorescence is observed in the solution.

C. Dissolve 0.1 g in 1 ml of acetone R and pour onto a clear glass plate; as the solvent evaporates, a glossy, clear film remains.

Free acid. Shake 1.0 g of finely powdered material with 100 ml of carbon-dioxide-free water R for 5 minutes and filter. Wash the flask and the filter with two quantities, each of 10 ml, of carbon-dioxide-free water R. Combine the filtrate and washings, add 0.1 ml of phenolphthalein/ethanol TS, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS until a faint pink colour is obtained. Repeat the procedure without the Cellacefate being examined and make any necessary corrections.

Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 8.306 mg of phthalic acid. Not more than 60 mg/g (6.0%) is found, calculated as phthalic acid and with reference to the anhydrous substance.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g and 20 ml of a mixture of equal volumes of dehydrated methanol R and chloroform R; the water content is not more than 50 mg/g (5.0%).

Assays

A. ***Phthaloyl groups.*** Dissolve about 0.4 g, accurately weighed, in 20 ml of ethylene glycol monomethyl ether R previously neutralized using 0.1 ml of phenolphthalein/ethanol TS. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS until a faint pink colour is obtained.

Calculate the content of phthaloyl groups in %: $\frac{149n}{(100-a)m} - 1.795S$

where n is the number of ml of carbonate-free sodium hydroxide (0.1 mol/l) VS used, a is the content of water in %, m is the mass of Cellacefate in g, and S is the content of free acid in %.

B. ***Acetyl groups.*** To about 0.1 g, accurately weighed, add 25 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS and heat on a water-bath under a reflux condenser for 30 minutes. Cool, add 0.1 ml of phenolphthalein/ethanol TS, and titrate with hydrochloric acid (0.1 mol/l) VS until the colour is discharged. Repeat the procedure without the Cellacefate being examined and make any necessary corrections.

Calculate the content of acetyl groups in %: $\frac{43(n_2-n_1)}{(100-a)m} - (0.578P + 0.518S)$

where n_2 is the number of ml of hydrochloric acid (0.1 mol/l) VS used for the blank, n_1 is the number of ml of hydrochloric acid (0.1 mol/l) VS used for Cellacefate, a is the content of water in %, m is the mass of Cellacefate in g, P is the content of phthaloyl groups in %, and S is the content of free acid in %.

CELLULOSUM MICROCRYSTALLINUM
MICROCRYSTALLINE CELLULOSE

Chemical name. Cellulose; CAS Reg. No. 9004-34-6.

Description. A white or almost white, fine crystalline or granular powder; odourless.

Solubility. Practically insoluble in water and most organic solvents; slightly soluble in dilute solutions of sodium hydroxide.

Category. Tablet and capsule diluent; suspending agent; disintegrant.

Storage. Microcrystalline cellulose should be kept in a well-closed container.

Additional information. Microcrystalline cellulose is usually defined by its particle size which ranges between 20 and 150 µm.

Requirements

Definition. Microcrystalline cellulose is partially depolymerized cellulose prepared from alpha cellulose.

Identity tests

A. Place 20 g on an air-jet sieve with a screen having a nominal aperture of 38 µm and shake for 5 minutes. If more than 1 g is retained on the screen, mix 30 g with 270 ml of water; otherwise, mix 45 g with 255 ml of water. Perform the mixing in a high-speed blender (18 000 rev/min) for 5 minutes. Transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours; a white, opaque, bubble-free dispersion is obtained without any supernatant liquid.

B. Dissolve 0.05 g in 10 ml of copper tetramine hydroxide TS; it dissolves completely without any residue. Add 5 ml of ethanol (~750 g/l) TS; a precipitate is produced.

Heavy metals. To 1.0 g add 4 ml of magnesium sulfate/sulfuric acid TS, mix, and heat cautiously to dryness on a water-bath. Progressively heat to ignition, not exceeding a temperature of 800 °C, and continue to heat until a white to greyish residue is obtained. Moisten the residue with 1 drop of hydrochloric acid (~250 g/l) TS and continue as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Water-soluble substances. Shake 5 g with 80 ml of water for 10 minutes. Filter into a tared dish, evaporate to dryness on a water-bath, dry at 105 °C for 1 hour, and weigh; the residue weighs not more than 2.0 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 60 mg/g.

pH value. Shake 2 g with 100 ml of carbon-dioxide-free water R for 5 minutes; pH of the supernatant liquid, 5.0–7.5.

Organic impurities. Place 10 mg on a watch-glass and add 0.05 ml of a freshly prepared solution of 0.1 g of phloroglucinol R in 5 ml of hydrochloric acid (~420 g/l) TS; no red colour appears.

Starch and dextrins. Shake 0.1 g with 5 ml of water and add 0.2 ml of iodine (0.05 mol/l) VS; no blue or red-brown colour develops.

CERA CARNAUBA
CARNAUBA WAX

Chemical name. Carnauba wax; CAS Reg. No. 8015-86-9.

Description. Pale yellow to light brown, moderately coarse powder, flakes, or irregular lumps of hard, brittle wax; odour, characteristic and free from rancidity.

Solubility. Practically insoluble in water; soluble in toluene R; slightly soluble in boiling ethanol (~750 g/l) TS.

Category. Polishing agent for coated tablets; viscosity-increasing agent for ointments; release-rate modifier for oral formulations.

Storage. Carnauba wax should be kept in a well-closed container.

Requirements

Definition. Carnauba wax is obtained from the leaves of *Copernicia cerifera* Mart. (Fam. Palmae).

Melting range. 78–85 °C.

Ash. Weigh 2.0 g and use an open porcelain or platinum dish. Heat over a flame; it volatilizes without emitting an acrid odour. Ignite; the residue weighs not more than 2.5 mg/g.

Acid value. Use 3 g; not more than 8.

Saponification value. Use about 3 g, accurately weighed, add 25 ml of xylene R, and dissolve by warming. To this solution add 50 ml of ethanol (~750 g/l) TS and proceed with the determination of saponification. Attach a reflux condenser and heat for 2 hours; 75–95.

Iodine value. 5–14.

CERA CETYLA
CETYL ESTERS WAX

Chemical name. C_{14-18} Fatty acids C_{14-18} alkyl esters; CAS Reg. No. 85566-24-1.

Other name. Synthetic spermaceti.

Description. White to almost white, somewhat translucent flakes (5 µm to several millimetres in the largest dimension), with a crystalline structure and a pearly lustre when caked; odour, faint, mild, aromatic.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in ether R; slightly soluble in hexane R.

Category. Stiffening agent.

Storage. Cetyl esters wax should be kept in a well-closed container, protected from heat.

Additional information. Cetyl esters wax has a mass density of about 0.83 g/ml at 50 °C.

Requirements

Definition. Cetyl esters wax is a mixture consisting primarily of esters of saturated fatty alcohols (C_{14} to C_{18}) and saturated fatty acids (C_{14} to C_{18}).

Melting range. 43–47 °C.

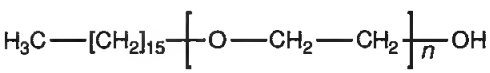
Acid value. Not more than 5.

Iodine value. Not more than 1.

Saponification value. 109–120.

Paraffin. To 1 g add 50 ml of boiling ethanol (~750 g/l) TS; the wax is completely dissolved.

CETOMACROGOLUM 1000
CETOMACROGOL 1000



$(C_2H_4O)nC_{16}H_{34}O$

Chemical name. Polyethylene glycol monohexadecyl ether; α-hexadecyl-ω-hydroxypoly(oxy-1,2-ethanediyl); CAS Reg. No. 9004-95-9.

Description. A cream-coloured, waxy, unctuous mass, pellets, or flakes; when heated, it melts to a brownish yellow, clear liquid; odourless or almost odourless.

Solubility. Soluble in water, ethanol (~750 g/l) TS, and acetone R; practically insoluble in light petroleum R.

Category. Nonionic surfactant.

Storage. Cetomacrogol 1000 should be kept in a well-closed container, protected from heat.

Requirements

Definition. Cetomacrogol 1000 is a condensation product of linear fatty alcohols with ethylene oxide, prepared under controlled conditions in order to obtain the required ether with the polyethylene glycol of the desired molecular mass.

Identity tests

A. Dissolve 0.1 g in 5 ml of water and add 10 ml of hydrochloric acid (~70 g/l) TS, 10 ml of barium chloride (50 g/l) TS, and 10 ml of phosphomolybdic acid (80 g/l) TS; a greenish yellow precipitate is produced.

B. Dissolve 0.1 g in 5 ml of water and add gradually tannic acid (50 g/l) TS; a precipitate is formed which dissolves on further addition of tannic acid solution.

Melting point. Not lower than 38 °C.

Refractive index. At 60 °C, $n_D^{20} = 1.448 - 1.452$.

Acid value. Not more than 0.5.

Alkalinity. Dissolve 2 g in 20 ml of carbon-dioxide-free water R, add 1 drop of phenolphthalein/ethanol TS, and titrate with hydrochloric acid (0.1 mol/l) VS; not more than 0.5 ml is required to obtain a pink colour.

Hydroxyl value. Use 10 g, Method A; 40.0–52.5.

Saponification value. Use 10 g; not more than 1.0.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 2.5 g; the water content is not more than 10 mg/g.

CETRIMIDUM
CETRIMIDE

Chemical name. Trimethyltetradecylammonium bromide mixture with dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide; cetrimide; CAS Reg. No. 8044-71-1.

Description. A white or almost white, voluminous, free-flowing powder; odour, slight, characteristic.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antimicrobial preservative.

Storage. Cetrimide should be stored in a well-closed container.

Requirements

Definition. Cetrimide is a mixture consisting mainly of tetradecyltrimethylammonium bromide together with smaller amounts of dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide.

Cetrimide contains not less than **96.0%** and not more than the equivalent of **101.0%** of alkyltrimethylammonium bromides, calculated as $C_{17}H_{38}BrN$ (relative molecular mass = 336.4) and with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 5 ml of phosphate buffer, pH 8.0, TS. Dip a strip of methyl green/iodomercurate paper R into the solution. Similarly prepare a blank solution without the Cetrimide being examined. After 5 minutes withdraw the strip of paper from the tube; the solution to be tested shows a darker greenish blue colour than the blank solution.

B. Dissolve 0.2 g in 10 ml of carbon-dioxide-free water R and shake; a voluminous froth is produced. (Keep the mixture for test C.)

C. The solution prepared above yields reaction A described under 2.1 General identification tests as characteristic of bromides.

Amines and amine salts. Dissolve 5 g in 30 ml of a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and add 100 ml of 2-propanol R. Slowly pass a stream of nitrogen R through the solution. Gradually add 15 ml of tetrabutylammonium hydroxide (0.1 mol/l) VS and titrate

potentiometrically, recording a titration curve; the volume of titrant added between the two points of inflexion is not larger than 2.0 ml.

Sulfated ash. Not more than 5.0 mg/g.

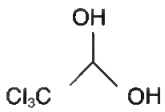
Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 20 mg/g.

Acidity or alkalinity. Dissolve 1 g in 50 ml of carbon-dioxide-free water R and add 0.1 ml of bromocresol purple/ethanol TS; not more than 0.1 ml of hydrochloric acid (0.1 mol/l) VS or 0.1 ml of sodium hydroxide (0.1 mol/l) VS is required to obtain the midpoint of the indicator (grey).

Assay. Dissolve about 2 g, accurately weighed, in 100 ml of water. Transfer 25 ml to a separating funnel, add 25 ml of chloroform R, 10 ml of sodium hydroxide (0.1 mol/l) VS, and 10 ml of a freshly prepared solution containing 5 g of potassium iodide R in 100 ml of water. Shake well, allow to separate, and discard the chloroform layer. Shake the aqueous layer with three quantities, each of 10 ml, of chloroform R, and discard the chloroform layers. Add 40 ml of hydrochloric acid (~420 g/l) TS, allow to cool, and titrate with potassium iodate (0.05 mol/l) VS until the deep brown colour is almost discharged. Add 2 ml of chloroform R and continue the titration, shaking vigorously, until the colour of the chloroform layer no longer changes. Repeat the procedure with a mixture of 10 ml of the above freshly prepared solution of potassium iodide, 20 ml of water, and 40 ml of hydrochloric acid (~420 g/l) TS and make any necessary corrections.

Each ml of potassium iodate (0.05 mol/l) VS is equivalent to 33.64 mg of $C_{17}H_{38}BrN$.

CHLORALI HYDRAS
CHLORAL HYDRATE



$C_2H_3Cl_3O_2$

Relative molecular mass. 165.4

Chemical name. 2,2,2-Trichloroethane-1,1-diol; CAS Reg. No. 302-17-0.

Description. Colourless, transparent or white crystals; odour, aromatic, pungent and characteristic.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Premedication.

Storage. Chloral hydrate should be kept in a tightly closed container.

Additional information. Melting temperature, about 55 °C; when exposed to air it slowly volatilizes.

Requirements

Chloral hydrate contains not less than **98.5%** and not more than **101.0%** of $C_2H_3Cl_3O_2$.

Note: Prepare the following test solution for use in "Identity tests A and B", and for "Clarity and colour". Dissolve 2.5 g in sufficient carbon-dioxide-free water R to produce 25 ml.

Identity tests

A. To 1.0 ml of the test solution add 2.0 ml of sodium sulfide TS; a yellow colour develops which quickly becomes reddish brown. On standing, a red precipitate may be produced.

B. Transfer 10 ml of the test solution to a conical flask and add 10 ml of 1-ethylquinaldinium iodide (15 g/l) TS that has previously been filtered through a 0.45-µm filter. Then add 60 ml of 2-propanol R, 5 ml of monoethanolamine (0.1 mol/l) VS, and 15 ml of water. Mix, and heat in a water-bath at 60 °C for 15 minutes; a blue colour develops.

Chlorides. Dissolve 2.5 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.1 mg/g.

Chloral alcoholate. Warm 1.0 g with 10 ml of sodium hydroxide (~80 g/l) TS. Filter the upper layer and add iodine (0.05 mol/l) VS a drop at a time until a yellow colour is obtained; no precipitate is produced within 1 hour.

Clarity and colour of solution. The test solution is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 3.5–5.5.

Assay. Dissolve about 4 g, accurately weighed, in 10 ml of carbon-dioxide-free water R and add 30.0 ml of carbonate-free sodium hydroxide (1 mol/l) VS. Allow the mixture to stand for 2 minutes and titrate with sulfuric acid (0.5 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure without the Chloral hydrate being examined and make any necessary corrections.

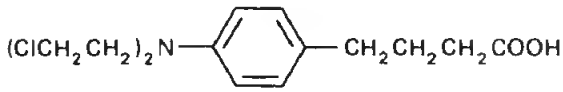
Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

CHLORAMBUCILUM
CHLORAMBUCIL

Molecular formula. $C_{14}H_{19}Cl_2NO_2$

Relative molecular mass. 304.2

Graphic formula.



Chemical name. 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyric acid; 4-[bis(2-chloroethyl)amino]benzenebutanoic acid; CAS Reg. No. 305-03-3.

Description. A white or almost white, crystalline or slightly granular powder.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS and acetone R.

Category. Cytotoxic drug.

Storage. Chlorambucil should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Chlorambucil must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Chlorambucil contains not less than 98.0% and not more than 101.0% of $C_{14}H_{19}Cl_2NO_2$, calculated with reference to the anhydrous substance.

Identity tests

A. Place 20 mg in a test-tube, add 0.20 ml of potassium dichromate TS2, cover the tube with a piece of filter-paper moistened with sodium nitroprusside (8.5 g/l) TS and 0.05 ml of piperidine R. Heat the tube over a small flame; a blue spot appears on the filter-paper.

B. Dissolve 0.05 g in 5 ml of acetone R, and dilute with water to 10 ml. Add 0.05 ml of sulfuric acid (~100 g/l) TS, then add 0.20 ml of silver nitrate (0.1 mol/l) VS; no opalescence is observed immediately (absence of chloride ion). Warm the solution on a water-bath; an opalescence develops (presence of ionizable chlorine).

C. Mix 0.4 g with 10 ml of hydrochloric acid (~70 g/l) TS and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with 2 quantities, each of 10 ml of water, and dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 3 hours; melting temperature, about 146 °C.

Melting range. 64–69 °C.

Sulfated ash. Not more than 1.0 mg/g.

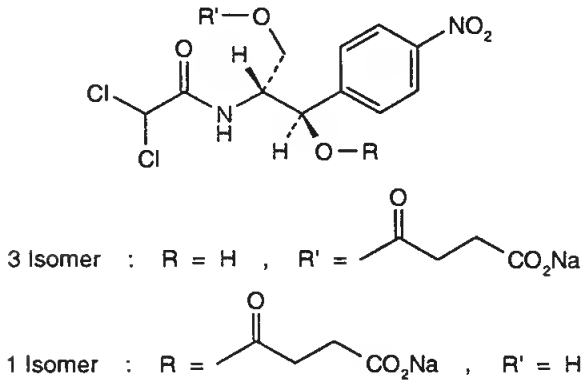
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

Related substance. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and allowing the coated plate to dry at room temperature for 24 hours. Use as the mobile phase a mixture of 8 volumes of toluene R, 5 volumes of methanol R, 4 volumes of heptane R, and 4 volumes of ethylmethylketone R. Apply separately to the plate 10 µl of each of 2 solutions in acetone R containing (A) 20 mg of the test substance per ml and (B) 0.40 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.2 g, accurately weighed, in 10 ml of acetone R, add 10 ml of water, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS using phenolphthalein/ethanol TS as indicator. Repeat the operation without

the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 30.42 mg of $C_{14}H_{19}Cl_2NO_2$.

CHLORAMPHENICOLI NATRII SUCCINAS
CHLORAMPHENICOL SODIUM SUCCINATE



$C_{15}H_{15}Cl_2N_2NaO_8$

Relative molecular mass. 445.2

Chemical name. A mixture in variable proportions of (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl succinate (3 isomer) and of sodium (1*R*,2*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-1-(4-nitrophenyl) propyl succinate (1 isomer); [*R*-(*R**,*R**)]-mono[2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl] ester, butanedioic acid, monosodium salt; D-*threo*-(-)-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl] acetamide α-(sodium succinate); CAS Reg. No. 982-57-0.

Description. A white or yellowish white powder.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS.

Category. Antibacterial drug.

Storage. Chloramphenicol sodium succinate should be kept in a tightly closed container, protected from light.

Labelling. The designation Chloramphenicol sodium succinate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. Chloramphenicol sodium succinate is hygroscopic. Even in the absence of light, Chloramphenicol sodium succinate gradually degrades when exposed to a humid atmosphere; decomposition is more rapid at higher temperatures.

Requirements

Chloramphenicol sodium succinate contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{15}Cl_2N_2NaO_8$, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from chloramphenicol sodium succinate RS or with the *reference spectrum* of chloramphenicol sodium succinate.

B. See the test described below under "Chloramphenicol and chloramphenicol disodium disuccinate, test B". The two principal spots obtained with solution A correspond in position and appearance with those obtained with solution B. The positions of the spots obtained with solutions A and B are different from that of the principal spot obtained with solution C.

C. Dissolve 10 mg in 2.0 ml of ethanol (~750 g/l) TS, add 0.2 g of zinc R powder, 1.0 ml of sulfuric acid (~100 g/l) TS, and allow to stand for 10 minutes. Filter. To the filtrate add 0.5 ml of sodium nitrite (10 g/l) TS, and allow to stand for 2 minutes. Then add 1.0 g of urea R and a solution containing 10 mg of 2-naphthol R in 2 ml of sodium hydroxide (~80 g/l) TS; a red colour is produced. Repeat the test omitting the zinc R powder; no red colour is produced.

D. Dissolve 5 mg in 5 ml of water and add a few drops of silver nitrate (40 g/l) TS; no precipitate is produced. Heat 0.05 g with 2.0 ml of potassium hydroxide/ethanol TS1 on a water-bath for 15 minutes, add 15 mg of charcoal R, shake, and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

E. When tested for sodium as described under 2.1 General identification tests, it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +5.0 ° to +8.0 °C.

Clarity of solution. A solution of 1.0 g in 3.0 ml of carbon-dioxide-free water R is clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of Chloramphenicol sodium succinate; the water content is not more than 0.20 g/g.

pH value. pH of a 0.25 g/ml solution in carbon-dioxide-free water R, 6.4–7.0.

Chloramphenicol and chloramphenicol disodium disuccinate

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 55 volumes of water, 40 volumes of methanol R, and 5 volumes of phosphoric acid (~20 g/l) TS.

   Prepare the following solutions in the mobile phase: solution (A) 0.25 mg of Chloramphenicol sodium succinate per ml; solution (B) 5.0 µg of chloramphenicol RS per ml; solution (C) 5.0 µg of chloramphenicol disodium disuccinate RS per ml; and for solution (D) dissolve 25 mg of Chloramphenicol sodium succinate in the mobile phase, add 0.5 mg of chloramphenicol RS and 0.5 mg of chloramphenicol disodium disuccinate RS and dilute to 100 ml with the mobile phase.

   Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 275 nm.

   Using a 20-µl loop injector inject solution D. Inject alternately solutions A, B, C, and D. The test is not valid unless the two peaks in the chromatogram obtained with solution D, corresponding to those in the chromatograms obtained with solutions B and C, are clearly separated from the peaks corresponding to the two principal peaks in the chromatogram obtained with solution A. If necessary, adjust the methanol content of the mobile phase.

   Measure the areas of the peak responses obtained in the chromatograms from solutions A, B, and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to chloramphenicol is not greater than that of the principal peak obtained with solution B (2.0%). The area of any peak corresponding to chloramphenicol disodium disuccinate is not greater than that of the principal peak obtained with solution C (2.0%).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 85 volumes of dichloromethane R, 14 volumes of methanol R, and 1 volume of acetic acid (~60 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 3 solutions in acetone R containing (A) 10 mg of Chloramphenicol sodium succinate per ml, (B) 10 mg of chloramphenicol sodium succinate RS per ml, and (C) 10 mg of chloramphenicol RS per ml. Then apply separately 10 µl of solution (A) as prepared above and 1 µl of solution (D) containing 0.20 mg of chloramphenicol RS per ml of acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with the second application of solution A, other than the principal spot, is not more intense than that obtained with solution D (2.0%).

Assay. Dissolve about 0.2 g, accurately weighed, in sufficient water to produce 500 ml; dilute 5.0 ml of this solution to 100 ml with water. Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 276 nm and calculate the percentage content of $C_{15}H_{15}Cl_2N_2NaO_8$ using the absorptivity value of 22.0 ($A^{1\%}_{1\,cm}$ = 220), and with reference to the anhydrous substance.

Additional requirements for Chloramphenicol sodium succinate for parenteral use

Complies with the monograph for "Parenteral preparations".

Storage. Sterile Chloramphenicol sodium succinate should be kept in a sterile, tightly closed, and tamper-evident container, protected from light.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.2 IU of endotoxin RS per mg.

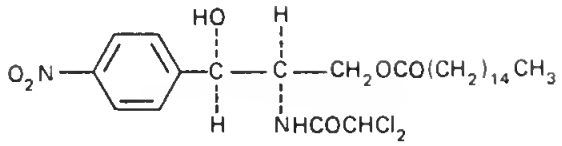
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, Membrane filtration test procedure.

CHLORAMPHENICOLI PALMITAS
CHLORAMPHENICOL PALMITATE

Molecular formula. $C_{27}H_{42}Cl_2N_2O_6$

Relative molecular mass. 561.5

Graphic formula.



Chemical name. D-*threo*-(-)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide α-palmitate; [*R*-(*R**,*R**)]-2-[(dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl hexadecanoate; CAS Reg. No. 530-43-8.

Description. A fine, white, unctuous, crystalline powder; odour, faint.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS; soluble in ether R.

Category. Antibacterial drug.

Storage. Chloramphenicol palmitate should be kept in a tightly closed container, protected from light.

Additional information. If Chloramphenicol palmitate is to be used to prepare a dosage form in the solid state, it should contain at least 90% of polymorph B. If a liquid dosage form is to be prepared, e.g., a suspension, the method of preparation should be such as to ensure that at least 90% of the chloramphenicol palmitate is present as polymorph B in the final product.

Requirements

Definition. Chloramphenicol palmitate contains not less than 98.0% and not more than 102.0% of $C_{27}H_{42}Cl_2N_2O_6$, calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of chloroform R, 1 volume of methanol R, and 0.1 volume of water as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in acetone R containing (A) 10 mg of the test substance per ml and (B) 10 mg of chloramphenicol palmitate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. Dissolve 10 mg in 4 ml of ethanol (~750 g/l) TS, add 1.0 ml of sulfuric acid (~100 g/l) TS and 0.05 g of zinc R powder, and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of sodium nitrite (100 g/l) TS and, after 2 minutes, 1.0 g of urea R, followed by 1.0 ml of 2-naphthol TS1 and 2.0 ml of sodium hydroxide (~400 g/l) TS; a red colour develops. Repeat the test omitting the zinc R powder; no red colour is produced.

Specific optical rotation. Use a 50 mg/ml solution in dehydrated ethanol R; $[\alpha]_D^{20\,°C} = +22.5$ to $+25.5°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 5.0 mg/g.

Acidity. Dissolve 1.0 g by warming to 35 °C with 5 ml of a mixture of equal volumes of ethanol (~750 g/l) TS and ether R, previously neutralized to phenolphthalein/ethanol TS. Titrate with sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator, until, on gentle shaking, a pink colour persists for 30 seconds; not more than 0.4 ml is required.

Free chloramphenicol. Dissolve, with the aid of gentle heat, 1.0 g in 80 ml of xylene R, cool, and extract with 3 quantities, each of 15 ml, of water; discard the xylene and dilute the combined aqueous extracts to 50 ml with water. Extract the solution with 10 ml of carbon tetrachloride R, allow to separate, discard the carbon tetrachloride, and centrifuge a portion of the aqueous solution. Measure the absorbance of the clear supernatant liquid in a 1-cm layer at the maximum at about 278 nm, using as the blank a solution obtained by repeating the procedure without the substance being examined; the absorbance of this blank solution should not exceed 0.05. Calculate the content of free chloramphenicol, using the absorptivity value of 29.8 ($A_{1\,cm}^{1\%} = 298$); not more than 0.45 mg/g.

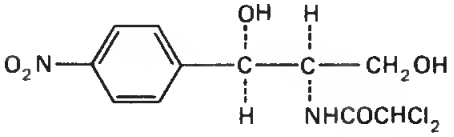
Assay. Dissolve about 0.03 g, accurately weighed, in sufficient dehydrated ethanol R to produce 100 ml; dilute 10 ml of this solution to 100 ml with the same solvent. Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 271 nm and calculate the content of $C_{27}H_{42}Cl_2N_2O_6$ using the absorptivity value of 17.8 ($A_{1\,cm}^{1\%} = 178$).

CHLORAMPHENICOLUM
CHLORAMPHENICOL

Molecular formula. $C_{11}H_{12}Cl_2N_2O_5$

Relative molecular mass. 323.1

Graphic formula.



Chloramphenicol

Chemical name. D-*threo*-(-)-2,2-Dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide; [*R*-(*R**,*R**)]-2,2-dichloro-*N*-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide; CAS Reg. No. 56-75-7.

Description. Colourless to greyish white or yellowish white, needle-like crystals or elongated plates or a crystalline powder; odourless.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS and propylene glycol R; slightly soluble in ether R.

Category. Antibiotic.

Storage. Chloramphenicol should be kept in a well-closed container, protected from light.

Additional information. Chloramphenicol has a bitter taste. A solution in dehydrated ethanol R is dextrorotatory and a solution in ethyl acetate R is levorotatory.

Requirements

Definition. Chloramphenicol contains not less than 97.0% and not more than 102.0% of $C_{11}H_{12}Cl_2N_2O_5$ calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from chloramphenicol RS or with the *reference spectrum* of chloramphenicol.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Melting temperature, about 151 °C.

Specific optical rotation. Use a 50 mg/ml solution in dehydrated ethanol R; $[\alpha]_D^{20\,°C} = +18.5$ to $+21.5$ °.

Free chlorides. For the preparation of the test solution shake 0.50 g with 20 ml of water and 10 ml of nitric acid (~130 g/l) TS for 1 minute, filter, and wash the filter with 5 ml of water. Proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.5 mg/g.

Solution in ethanol. A solution of 0.50 g in 10 ml of ethanol (~750 g/l) TS is clear.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. Shake 0.05 g with 10 ml of carbon-dioxide-free water R; pH of the suspension, 5.0–7.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of 3 freshly prepared solutions in ethanol (~750 g/l) TS containing (A) 20 mg of the test substance per ml, (B) 0.20 mg of the test substance per ml, and (C) 0.20 mg of chloramphenicol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 105 °C for 5 minutes, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in sufficient water to produce 100 ml; dilute 10.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 278 nm. Calculate the amount of $C_{11}H_{12}Cl_2N_2O_5$ in the substance being

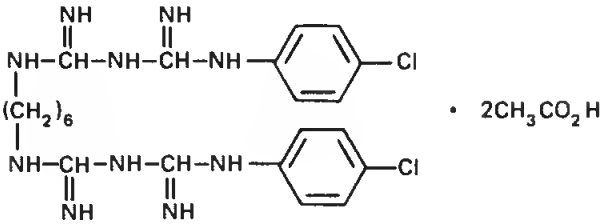
tested by comparison with chloramphenicol RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer, the absorbance of the reference solution should be 0.60 ± 0.03.

CHLORHEXIDINI DIACETAS
CHLORHEXIDINE DIACETATE

Molecular formula. $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$

Relative molecular mass. 625.6

Graphic formula.



Chemical name. 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] diacetate; *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide diacetate; CAS Reg. No. 56-95-1.

Description. A white or yellowish white, microcrystalline powder; odourless or almost odourless.

Solubility. Soluble in 55 parts of water and in 15 parts of ethanol (~750 g/l) TS; very slightly soluble in glycerol R.

Category. Disinfectant.

Storage. Chlorhexidine diacetate should be kept in a well-closed container, protected from light.

Requirements

Definition. Chlorhexidine diacetate contains not less than 97.5% and not more than 101.0% of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.1 g in 10 ml of methanol R by warming, and add a mixture of 2 ml of sodium hydroxide (~150 g/l) TS and 2 ml of bromine TS1; a deep red colour is produced.

B. Dissolve 0.1 g in 10 ml of water and add, with shaking, 0.15 ml of copper(II) chloride/ammonia TS; a purple precipitate is produced immediately. Continue to add 0.5 ml of copper(II) chloride/ammonia TS; the colour of the precipitate changes to blue.

C. Heat gently 0.2 g with 1 ml of ethanol (~750 g/l) TS and 1 ml of sulfuric acid (~1760 g/l) TS; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 35 mg/g.

Chloraniline. Dissolve 0.20 g in 30 ml of water. Add with mixing 5 ml of hydrochloric acid (1 mol/l) VS, 1 ml of sodium nitrite (35 g/l) TS, 2 ml of ammonium sulfamate (50 g/l) TS, and shake. Then add 5 ml of freshly prepared *N*-(1-naphthyl)ethylenediamine hydrochloride(1 g/l) TS, 1 ml of ethanol (~750 g/l) TS, and sufficient water to produce 50 ml. Allow to stand for 30 minutes. Treat similarly 30 ml of a solution containing 0.10 mg of chloraniline R that has been slightly acidified with hydrochloric acid (~70 g/l) TS. The colour produced in the test solution is not more intense than that of the reference solution when compared as described in 1.11 Colour of liquids (0.5 mg/g).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and preparing a slurry as follows: To 8 g of silica gel R4 add 16 ml of water containing 1 g of sodium formate R and coat the plates with a layer 0.5 mm thick. Use a mixture of 50 volumes of chloroform R, 50 volumes of ethanol (~750 g/l) TS and 7 volumes of formic acid (~1080 g/l) TS as the mobile phase. Apply to the plate in the form of a band, 4 cm wide, 20 µl of a solution in acetic acid (~90 g/l) TS containing 72 mg of the test substance per ml (solution A). After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Score a rectangular area around each group of bands above and below the principal band, quantitatively transfer the enclosed areas of silica gel to a glass-stoppered test-tube, add 5 ml of methanol R, shake for 15 minutes, centrifuge, and measure the absorbance of the clear supernatant liquid in a 1-cm layer at the maximum at about 256 nm. For the blank solution treat in a similar manner equivalent sized areas of silica gel removed from the coating adjacent to the areas previously removed. Prepare solution B in the following manner: Dissolve 0.14 g of the test substance in suf-

ficient acetic acid (~90 g/l) TS to produce 100 ml and dilute 200 µl of this solution to 50 ml with methanol R. The absorbance obtained from the eluted solution A is not greater than the absorbance obtained from solution B.

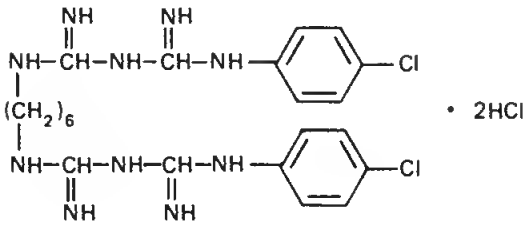
Assay. Dissolve about 0.45 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 15.64 mg of $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$.

CHLORHEXIDINI DIHYDROCHLORIDUM
CHLORHEXIDINE DIHYDROCHLORIDE

Molecular formula. $C_{22}H_{30}Cl_2N_{10},2HCl$

Relative molecular mass. 578.4

Graphic formula.



Chemical name. 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] dihydrochloride; *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide dihydrochloride; CAS Reg. No. 3697-42-5.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Sparingly soluble in water; soluble in 450 parts of ethanol (~750 g/l) TS.

Category. Disinfectant.

Storage. Chlorhexidine dihydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Chlorhexidine dihydrochloride contains not less than 98.0% and not more than 101.0% of $C_{22}H_{30}Cl_2N_{10},2HCl$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 20 mg in 10 ml of methanol R by warming, and add a mixture of 2 ml of sodium hydroxide (~150 g/l) TS and 2 ml of bromine TS1; a deep red colour is produced.

B. Dissolve 0.1 g in 10 ml of water and add, with shaking, 0.15 ml of copper(II) chloride/ammonia TS; a purple precipitate is produced immediately. Continue to add 0.5 ml of copper(II) chloride/ammonia TS; the colour of the precipitate changes to blue.

C. Dissolve 0.1 g in 50 ml of nitric acid (~130 g/l) TS; the solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 130 °C; it loses not more than 20 mg/g.

Chloraniline. Dissolve 0.20 g in 30 ml of water. Add with mixing 5 ml of hydrochloric acid (1 mol/l) VS, 1 ml of sodium nitrite (35 g/l) TS, 2 ml of ammonium sulfamate (50 g/l) TS and shake. Then add 5 ml of freshly prepared *N*-(1-naphthyl)ethylenediamine hydrochloride(1 g/l)TS, 1 ml of ethanol (~750 g/l) TS, and sufficient water to produce 50 ml. Allow to stand for 30 minutes. Treat similarly 30 ml of a solution containing 0.10 mg of chloraniline R that has been slightly acidified with hydrochloric acid (~70 g/l) TS. The colour produced in the test solution is not more intense than that of the reference solution when compared as described in 1.11 Colour of liquids (0.5 mg/g).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and preparing a slurry as follows: To 8 g of silica gel R4 add 16 ml of water containing 1 g of sodium formate R and coat the plates with a layer, 0.5 mm thick. Use a mixture of 50 volumes of chloroform R, 50 volumes of ethanol (~750 g/l) TS, and 7 volumes of formic acid (~1080 g/l) TS as the mobile phase. Prepare solution A by dissolving 1.1 g of the test substance in 35 ml of hydrochloric acid (~330 g/l) TS, add 100 ml of 2-propanol R, cool in ice, make alkaline with sodium hydroxide (~200 g/l) TS, cool in ice, add 200 ml of ice-cooled water, and extract with 100 ml of chloroform R. Dry the chloroform extract over anhydrous potassium carbonate R, filter, evaporate the chloroform almost to dryness under a stream

of nitrogen R, add 50 ml of methanol R, evaporate to dryness under a stream of nitrogen R, and dry the residue at 65 °C for 30 minutes; dissolve 0.56 g of the dried residue in sufficient acetic acid (~90 g/l) TS to produce 100 ml. Apply to the plate in the form of a band, 4 cm wide, 20 µl of solution A. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Score a rectangular area around each group of bands above and below the principal band, quantitatively transfer the enclosed areas of silica gel to a glass-stoppered test-tube, add 5 ml of methanol R, shake for 15 minutes, centrifuge, and measure the absorbance of the clear supernatant liquid in a 1-cm layer at the maximum at about 256 nm. For the blank solution treat in a similar manner equivalent-sized areas of silica gel removed from the coating adjacent to the areas previously removed. Prepare solution B in the following manner: Dissolve 0.11 g of the dried residue in sufficient acetic acid (~90 g/l) TS to produce 100 ml and dilute 200 µl of this solution to 50 ml with methanol R. The absorbance obtained from the eluted solution A is not greater than the absorbance obtained from solution B.

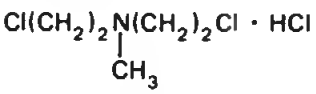
Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 14.46 mg of $C_{22}H_{30}Cl_2N_{10},2HCl$.

CHLORMETHINI HYDROCHLORIDUM
CHLORMETHINE HYDROCHLORIDE

Molecular formula. $C_5H_{11}Cl_2N,HCl$

Relative molecular mass. 192.5

Graphic formula.



Chemical name. 2,2′-Dichloro-*N*-methyldiethylamine hydrochloride; 2-chloro-*N*-(2-chloroethyl)-*N*-methylethanamine hydrochloride; CAS Reg. No. 55-86-7.

Description. A white or almost white, crystalline powder.

Solubility. Very soluble in water; soluble in ethanol (~750 g/l) TS.

Category. Antineoplastic.

Storage. Chlormethine hydrochloride should be kept in a tightly closed container and stored at a cool temperature.

Additional information. CAUTION: Chlormethine hydrochloride is vesicant, it must be handled with care avoiding contact with the skin and inhaling airborne particles. It is hygroscopic.

Requirements

Definition. Chlormethine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_5H_{11}Cl_2N,HCl$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 0.05 g in 5 ml of water and add 0.02 ml of potassio-mercuric iodide TS; a cream-coloured precipitate is produced.

B. Add 0.1 g to 1 ml of sodium thiosulfate (0.1 mol/l) VS contained in a test-tube. Shake, allow to stand for 2 hours, and add 1 drop of iodine TS; the colour of free iodine remains.

C. Melting temperature, about 110 °C with decomposition.

Clarity of solution. A solution of 0.10 g in 10 ml of carbon-dioxide-free water R is clear.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 5.0 mg/g.

pH value. pH of a 2.0 mg/ml solution, 3.0–5.0.

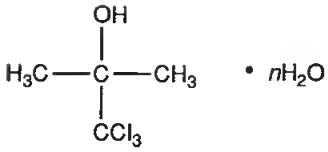
Assay. To about 0.20 g, accurately weighed, add 15 ml of potassium hydroxide/ethanol (1 mol/l) VS and 15 ml of water. Boil under a reflux condenser for 2 hours, and evaporate the solution to half its volume on a water-bath. Dilute to 150 ml with water, add 3 ml of nitric acid (~1000 g/l) TS and 50 ml of silver nitrate (0.1 mol/l) VS. Shake vigorously and filter. Wash the precipitate with water and titrate the excess of silver nitrate in the combined filtrate and washings with ammonium thiocyanate (0.1 mol/l) VS, using 2.5 ml of ferric ammo-

nium sulfate (45 g/l) TS as indicator. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 6.417 mg of $C_5H_{11}Cl_2N,HCl$.

CHLOROBUTANOLUM

CHLOROBUTANOL

Chlorobutanol, anhydrous
Chlorobutanol hemihydrate



$n = 0$ (anhydrous)
$n = \frac{1}{2}$ (hemihydrate)

$C_4H_7Cl_3O$ (anhydrous)
$C_4H_7Cl_3O,\frac{1}{2}H_2O$ (hemihydrate)

Relative molecular mass. 177.5 (anhydrous); 186.5 (hemihydrate).

Chemical name. 1,1,1-Trichloro-2-methyl-2-propanol; CAS Reg. No. 57-15-8 (anhydrous).

1,1,1-Trichloro-2-methyl-2-propanol hemihydrate; CAS Reg. No. 6001-64-5 (hemihydrate).

Description. Colourless crystals or a white, crystalline powder; odour, characteristic, camphoraceous.

Solubility. Slightly soluble in water; very soluble in ethanol (~750 g/l) TS and ether R; soluble in glycerol R.

Category. Antimicrobial preservative.

Storage. Chlorobutanol should be kept in a tightly closed container, and stored in a cool place.

Labelling. The designation on the container of Chlorobutanol should state whether it is the hemihydrate or the anhydrous form.

Additional information. Anhydrous Chlorobutanol melts at about 95 °C and Chlorobutanol hemihydrate melts at about 77 °C, both determined without previous drying.

Requirements

Chlorobutanol contains not less than **98.0%** and not more than the equivalent of **101.0%** of $C_4H_7Cl_3O$, calculated with reference to the anhydrous substance.

Identity tests

A. Shake 20 mg with 3 ml of sodium hydroxide (1 mol/l) VS, add 5 ml of water, then slowly add 2 ml of iodine TS; iodoform, perceptible by its odour, is produced and a yellowish precipitate is formed.

B. To 20 mg add 1 ml of pyridine R and 2 ml of sodium hydroxide (~400 g/l) TS. Heat in a water-bath and shake. Allow to stand; the pyridine layer becomes red.

Solution in ethanol. A solution of 5 g in 10 ml of ethanol (~750 g/l) TS is not more opalescent than opalescence standard TS2 and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

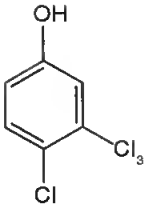
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. For the anhydrous form, use 2 g; the water content is not more than 10 mg/g. For the hemihydrate, use 0.3 g; the water content is not less than 45 mg/g and not more than 60 mg/g.

Acidity. Dissolve 2 g in 20 ml of ethanol (~750 g/l) TS and titrate with sodium hydroxide (0.01 mol/l) VS, using 0.1 ml of bromothymol blue/ethanol TS as indicator; not more than 1.0 ml is required to produce a blue colour.

Assay. Dissolve about 0.1 g, accurately weighed, in 20 ml of ethanol (~750 g/l) TS, add 10 ml of sodium hydroxide (~80 g/l) TS, heat in a water-bath for 5 minutes, and cool. Add 20 ml of nitric acid (~130 g/l) TS, 25.0 ml of silver nitrate (0.1 mol/l) VS, and 2 ml of dibutyl phthalate R, and shake vigorously. Add 2 ml of ferric ammonium sulfate (45 g/l) TS and titrate with ammonium thiocyanate (0.1 mol/l) VS until an orange colour is obtained. Repeat the procedure without the Chlorobutanol being examined and make any necessary corrections.

Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 5.916 mg of $C_4H_7Cl_3O$.

CHLOROCRESOLUM
CHLOROCRESOL



C_7H_7ClO

Relative molecular mass. 142.6

Chemical name. 4-Chloro-*m*-cresol; 4-chloro-3-methylphenol; CAS Reg. No. 59-50-7.

Description. Colourless or almost colourless crystals or a white, crystalline powder; odour, characteristic.

Solubility. Slightly soluble in water; very soluble in ethanol (~750 g/l) TS; freely soluble in ether R, fatty oils, and sodium hydroxide (~80 g/l) TS.

Category. Antimicrobial preservative.

Storage. Chlorocresol should be kept in a well-closed container, protected from light.

Requirements

Chlorocresol contains not less than **98.0%** and not more than the equivalent of **101.0%** of C_7H_7ClO.

Identity tests

A. To 0.5 g of a fine powder, add 10 ml of carbon-dioxide-free water R, shake for 2 minutes, and filter. Add 0.1 ml of ferric chloride (25 g/l) TS; a bluish colour is produced.

B. Mix 0.05 g with 0.5 g of anhydrous sodium carbonate R and ignite with a strong flame. Cool, add a mixture of 5 ml of water and 5 ml of nitric acid (~130 g/l) TS to the residue, and filter. Add 1 ml of silver nitrate (0.1 mol/l) VS to the filtrate; a white precipitate is produced.

Melting range. 64–67 °C.

Non-volatile residue. Place 2 g in a porcelain dish and heat on a water-bath until volatilized. Dry the residue at 105 °C and weigh; not more than 1.0 mg/g.

Assay. Place about 0.07 g, accurately weighed, in a ground-glass-stoppered flask and dissolve in 30 ml of glacial acetic acid R. Add 25 ml of potassium bromate (0.0167 mol/l) VS, a solution composed of 3 g of potassium bromide R dissolved in 20 ml of water, and 10 ml of hydrochloric acid (~420 g/l) TS. Allow to stand protected from light for 15 minutes. Add 1 g of potassium iodide R and 100 ml of water. Titrate with sodium thiosulfate (0.1 mol/l) VS, shaking vigorously, and add starch TS as indicator towards the end of the titration. Repeat the procedure without the Chlorocresol being examined and make any necessary corrections.

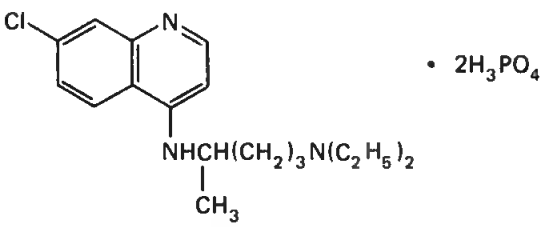
Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 3.565 mg of C_7H_7ClO.

CHLOROQUINI PHOSPHAS
CHLOROQUINE PHOSPHATE

Molecular formula. $C_{18}H_{26}ClN_3,2H_3PO_4$

Relative molecular mass. 515.9

Graphic formula.



Chemical name. 7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline phosphate (1:2); N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine phosphate (1:2); CAS Reg. No. 50-63-5.

Description. A white or almost white, crystalline powder: odourless or almost odourless.

Solubility. Soluble in 4 parts of water; very slightly soluble in ethanol (~750 g/l) TS and ether R.

Category. Antimalarial; antiamoebic.

Storage. Chloroquine phosphate should be kept in a well-closed container, protected from light.

Additional information. Chloroquine phosphate has a bitter taste; it is slowly discoloured by light. Chloroquine phosphate may exist in 2 polymorphic forms differing in melting temperature, one of which melts at about 194 °C, the other at about 215 °C; mixtures of the 2 forms melt between 194 °C and 215 °C.

Requirements

Definition. Chloroquine phosphate contains not less than 98.0% and not more than 101.0% of $C_{18}H_{26}ClN_3,2H_3PO_4$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 240 nm and 360 nm, exhibits 3 maxima at about 257 nm, 329 nm, and 343 nm. The absorbances at those wavelengths are about 0.29, 0.32, and 0.37, respectively (preferably use 2-cm cells for the measurements and calculate the absorbances of 1-cm layers). The ratio of the absorbance of a 1-cm layer at 257 nm to that at 343 nm is between 0.77 and 0.85, and the ratio of the absorbance at 329 nm to that at 343 nm is between 0.86 and 0.95.

B. To 1 ml of a 20 mg/ml solution add 3 ml of nitric acid (~130 g/l) TS; yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

C. To a solution of 0.05 g in 20 ml of water add 5 ml of trinitrophenol (7 g/l) TS. Filter, wash the precipitate with water until the filtrate is colourless, and dry the precipitate over silica gel, desiccant, R. Melting temperature, about 207 °C (picrate).

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

pH value. pH of a 0.10 g/ml solution, 3.5–4.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of chloroform R, 4 volumes of cyclohexane R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 40 mg of the test substance per ml and (B) 0.80 mg of the test substance per ml. After removing the plate from the chromatographic

chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.23 g, accurately weighed, in 20 ml of glacial acetic acid R1 with the aid of heat (preferably heat under reflux condenser), cool and add 20 ml of dioxan R. Titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.79 mg of $C_{18}H_{26}ClN_3,2H_3PO_4$.

Additional requirement for Chloroquine phosphate for parenteral use

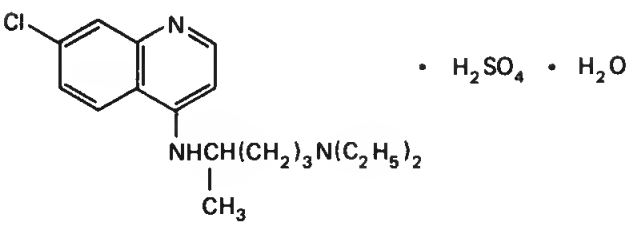
Complies with the monograph for "Parenteral preparations".

CHLOROQUINI SULFAS
CHLOROQUINE SULFATE

Molecular formula. $C_{18}H_{26}ClN_3,H_2SO_4,H_2O$

Relative molecular mass. 436.0

Graphic formula.



Chemical name. 7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline sulfate (1:1) monohydrate; N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine sulfate (1:1) monohydrate; CAS Reg. No. 6823-83-2.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 3 parts of water; practically insoluble in ethanol (~750 g/l) TS; sparingly soluble in ether R.

Category. Antimalarial.

Storage. Chloroquine sulfate should be kept in a well-closed container protected from light.

Additional information. Chloroquine sulfate has a bitter taste.

Requirements

Definition. Chloroquine sulfate contains not less than 98.0% and not more than 101.0% of $C_{18}H_{26}ClN_3,H_2SO_4$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 240 nm and 360 nm, exhibits 3 maxima at about 257 nm, 329 nm, and 343 nm. The absorbances at those wavelengths are about 0.39, 0.44, and 0.46, respectively (preferably use 2-cm cells for the measurements and calculate the absorbances of 1-cm layers). The ratio of the absorbance of a 1-cm layer at 257 nm to that at 343 nm is between 0.83 and 0.98 and the ratio of the absorbance at 329 nm to that at 343 nm is between 0.94 and 1.03.

B. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

C. To a solution of 0.05 g in 20 ml of water add 5 ml of trinitrophenol (7 g/l) TS. Filter, wash the precipitate with water until the filtrate is colourless and dry the precipitate over silica gel, desiccant, R. Melting temperature, about 207 °C (picrate).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not less than 30 mg/g and not more than 50 mg/g.

pH value. pH of a 0.10 g/ml solution, 4.0–5.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of chloroform R, 4 volumes of cyclohexane R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 40 mg of the test substance per ml and (B) 0.80 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 20 ml of glacial acetic acid R1 with the aid of heat (preferably under a reflux condenser), cool, and add 20 ml of dioxan R. Titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 41.8 mg of $C_{18}H_{26}ClN_3,H_2SO_4$.

Additional requirement for Chloroquine sulfate for parenteral use

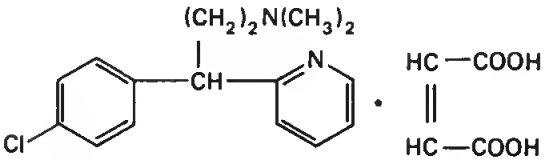
Complies with the monograph for "Parenteral preparations".

CHLORPHENAMINI HYDROGENOMALEAS
CHLORPHENAMINE HYDROGEN MALEATE

Molecular formula. $C_{16}H_{19}ClN_2,C_4H_4O_4$ or $C_{20}H_{23}ClN_2O_4$

Relative molecular mass. 390.9

Graphic formula.



Chemical name. 2-[*p*-Chloro-α-[2-(dimethylamino)ethyl]benzyl]pyridine maleate (1:1); γ-(4-chlorophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine (*Z*)-2-butenedioate (1:1); CAS Reg. No. 113-92-8.

Other name. Chlorpheniramine hydrogen maleate.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 4 parts of water; soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Antihistaminic.

Storage. Chlorphenamine hydrogen maleate should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Chlorphenamine hydrogen maleate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Chlorphenamine hydrogen maleate contains not less than 98.0% and not more than 101.0% of $C_{16}H_{19}ClN_2,C_4H_4O_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from chlorphenamine hydrogen maleate RS or with the *reference spectrum* of chlorphenamine hydrogen maleate.

B. Dissolve 1 mg in 5 ml of water. To 1 ml of this solution add 1 ml of buffer phthalate, pH 3.5, TS and 1 ml of cyanogen bromide TS. Allow to stand for 10 minutes with intermittent, gentle shaking. Add 2 ml of a solution composed of 1 ml of aniline R diluted to 25 ml with dichloroethane R, and shake; an orange-yellow colour is produced in the lower layer.

C. Dissolve 0.5 g in 5 ml of water, add 0.2 ml of sulfuric acid (~100 g/l) TS and extract 4 times with ether R, using 25 ml each time. Combine the ethereal extracts, dry them over anhydrous sodium sulfate R, filter, and evaporate the filtrate in a current of warm air; melting temperature of the residue, about 132 °C (maleic acid).

Sulfated ash. Not more than 1.5 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 20 mg/ml solution, 4.0–5.2.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of ethyl acetate R, 3 volumes of methanol R, and 2 volumes of acetic acid (~60 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in chloroform R containing (A) 50 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A,

other than the two principal spots due to chlorphenamine and maleic acid, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 19.54 mg of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Additional requirements for Chlorphenamine hydrogen maleate for parenteral use

Complies with the monograph for "Parenteral preparations".

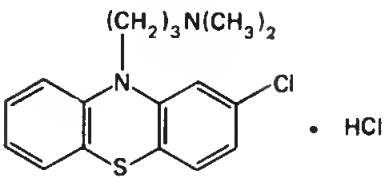
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 8.8 IU of endotoxin RS per mg.

CHLORPROMAZINI HYDROCHLORIDUM
CHLORPROMAZINE HYDROCHLORIDE

Molecular formula. $C_{17}H_{19}ClN_2S$, HCl

Relative molecular mass. 355.3

Graphic formula.



Chemical name. 2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine monohydrochloride; 2-chloro-*N*,*N*-dimethyl-10*H*-phenothiazine-10-propanamine monohydrochloride; CAS Reg. No. 69-09-0.

Description. A white or slightly creamy white, crystalline powder; odour, slight.

Solubility. Soluble in 0.4 part of water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Neuroleptic.

Storage. Chlorpromazine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Chlorpromazine hydrochloride has a very bitter taste. It darkens on prolonged exposure to light. Even in the absence of light, Chlorpromazine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperature.

Requirements

Definition. Chlorpromazine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{17}H_{19}ClN_2S,HCl$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or all 3 tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from chlorpromazine hydrochloride RS or with the *reference spectrum* of chlorpromazine hydrochloride.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of 2-phenoxyethanol R, 5 volumes of macrogol 400R, and 85 volumes of acetone R to impregnate the plate. After the solvent has reached the top of the plate, remove the plate from the chromatographic chamber, and use it immediately. As the mobile phase use a mixture of 2 volumes of diethylamine R and 100 volumes of light petroleum R1 saturated with 2-phenoxyethanol R. Apply separately to the plate 2 µl of each of 2 solutions in chloroform R containing (A) 2.0 mg of the test substance per ml and (B) 2.0 mg of chlorpromazine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm), observing the fluorescence produced after about 2 minutes. Spray the plate with sulfuric acid/ethanol TS and examine the chromatogram in daylight. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. A 0.1 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

D. Melting temperature, about 196 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a freshly prepared 0.10 g/ml solution, 4.0–5.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of cyclohexane R, 10 volumes of acetone R, and 10 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 freshly prepared solutions in a mixture of 95 volumes of methanol R and 5 volumes of diethylamine R containing (A) 20 mg of the test substance per ml and (B) 0.50 mg of the test substance per ml. After removing the plate from the chromatographic chamber allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Ignore any spot on the base line. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.7 g, accurately weighed, in 200 ml of acetone R, add 10 ml of mercuric acetate/acetic acid TS and 3 ml of methyl orange/acetone TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 35.53 mg of $C_{17}H_{19}ClN_2S,HCl$.

Additional requirements for Chlorpromazine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

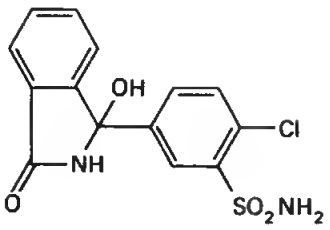
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 6.9 IU of endotoxin RS per mg.

CHLORTALIDONUM
CHLORTALIDONE

Molecular formula. $C_{14}H_{11}ClN_2O_4S$

Relative molecular mass. 338.8

Graphic formula.



Chemical name. 2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide; 2-chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1*H*-isoindol-1-yl)benzenesulfonamide; CAS Reg. No. 77-36-1.

Description. A white to yellowish white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water and ether R; soluble in methanol R; slightly soluble in ethanol (~750 g/l) TS.

Category. Diuretic.

Storage. Chlortalidone should be kept in a well-closed container.

Requirements

Definition. Chlortalidone contains not less than 98.0% and not more than 102.0% of $C_{14}H_{11}ClN_2O_4S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from chlortalidone RS or with the *reference spectrum* of chlortalidone.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 20 mg in 1 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced. Warm the mixture in a water-bath and add 10 mg of 1-naphthol R; a red-violet colour is produced.

Solution in alkali. A solution of 1.0 g in 10 ml of sodium hydroxide (~200 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 15 volumes of 1-butanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 10 mg of chlortalidone RS per ml, and (C) 0.10 mg of 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of pyridine R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 33.88 mg of $C_{14}H_{11}ClN_2O_4S$.

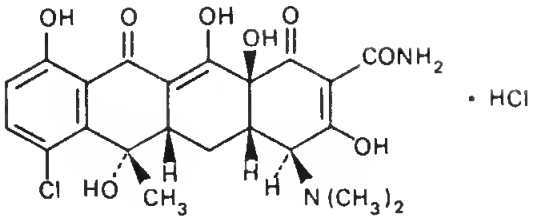
CHLORTETRACYCLINI HYDROCHLORIDUM
CHLORTETRACYCLINE HYDROCHLORIDE

Chlortetracycline hydrochloride (non-injectable)
Chlortetracycline hydrochloride, sterile

Molecular formula. $C_{22}H_{23}ClN_2O_8,HCl$

Relative molecular mass. 515.4

Graphic formula.



Chemical name. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; [4*S*-(4α,4aα,5aα,6β,12aα)]-7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-penta hydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; CAS Reg. No. 64-72-2.

Description. Yellow crystals or a yellow, crystalline powder; odourless.

Solubility. Soluble in about 100 parts of water and in about 250 parts of ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Antiinfective drug.

Storage. Chlortetracycline hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Chlortetracycline hydrochloride indicates that the substance complies with the additional requirements for sterile Chlortetracycline hydrochloride and may be used for parenteral administration or for other sterile applications.

Additional information. Chlortetracycline hydrochloride has a bitter taste. Even in the absence of light, Chlortetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Chlortetracycline hydrochloride contains not less than 900 International Units of Chlortetracycline per mg, calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a kieselguhr coating prepared as follows: To 25 g of kieselguhr R1 add 50 ml of a mixture of 2.5 ml of glycerol R and 47.5 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS. Coat the plates with this mixture, and allow them to dry at room temperature for about 70–90 minutes, or until sufficiently dry to give a satisfactory separation. As the mobile phase, take 200 ml of a mixture of 2 volumes of ethyl acetate R, 2 volumes of chloroform R, and 1 volume of acetone R, Shake with 25 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS, allow to settle, and use the lower layer. Apply separately to the plate 1 µl of each of 3 solutions in methanol R containing (A) 0.50 mg of the test substance per ml, (B) 0.50 mg of chlortetracycline hydrochloride RS per ml, and (C) a mixture of 0.50 mg of chlortetracycline hydrochloride RS per ml, 0.50 mg of oxytetracycline hydrochloride RS per ml, and 0.50 mg of tetracycline hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows 3 clearly separated spots.

B. Dissolve 10 mg in 10 ml of phosphate buffer, pH 7.6, TS, heat at 100 °C for 1 minute and examine the solution in ultraviolet light (365 nm); a strong blue fluorescence is observed.

C. To about 1 mg add 2 ml of sulfuric acid (~1760 g/l) TS; a blue to bluish green colour is produced, which changes to brown on the addition of about 1 ml of water.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Dissolve 0.125 g in sufficient water to produce 25.0 ml, and allow to stand in the dark for 30 minutes. Measure the rotation at 25 °C and calculate with reference to the dried substance; $[\alpha]_D^{25\,°C} = -235$ to -250 °.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 50 µg/g.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 20 mg/g.

pH value. pH of a 10 mg/ml solution, 2.3–3.3.

Absorption in the ultraviolet region. Dissolve 10 mg in sufficient sulfuric acid (0.5 mol/l) VS to produce 100 ml. Dilute 10 ml of this solution to 100 ml with sulfuric acid (0.5 mol/l) VS. Place 10 ml of the resulting solution in a test-tube 25 mm in diameter and 200 mm long and immerse in a water-bath for 8 minutes. Cool, replace the water lost by evaporation, and measure the absorbance of a 1-cm layer at 274 nm; not less than 0.70 and not more than 0.76.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5–6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of chlortetracycline (usually between 2 and 20 IU per ml), and an incubation temperature of 35–39 °C, or (*b*) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9–6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of chlortetracycline (usually between 0.05 and 0.2 IU), and an incubation temperature of 29–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 900 IU of chlortetracycline per mg, calculated with reference to the dried substance.

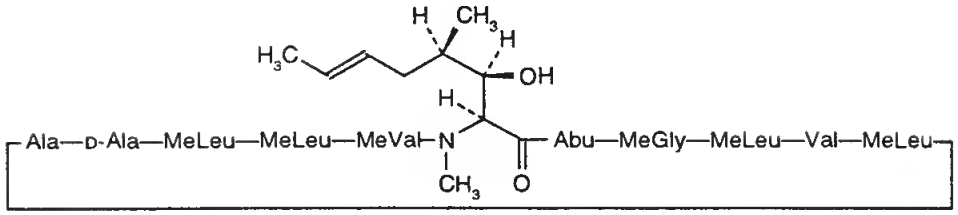
Additional requirements for Chlortetracycline Hydrochloride for sterile use

Storage. Sterile Chlortetracycline hydrochloride should be kept in a hermetically closed container, protected from light.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.0 IU of endotoxin RS per mg.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

CICLOSPORINUM
CICLOSPORIN



$C_{62}H_{111}N_{11}O_{12}$

Relative molecular mass. 1203

Chemical name. Cyclo[[(*E*)-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*methyl-L-valyl]; cyclosporin A; CAS Reg. No. 59865-13-3.

Other name. Cyclosporin.

Description. A white or almost white powder.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS and dichloromethane R.

Category. Immunosuppressant drug.

Storage. Ciclosporin should be kept in a well-closed container, protected from light.

Requirements

Ciclosporin contains not less than **98.5%** and not more than **101.5%** of $C_{62}H_{111}N_{11}O_{12}$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ciclosporin RS or with the *reference spectrum* of ciclosporin.

B. See the test described below under "Related substances" and under "Assay". The principal peak obtained with solution A corresponds in retention time to that obtained with solution B.

C. Dissolve 5 mg in 5 ml of methanol R, and 1 drop of potassium permanganate (10 g/l) TS, and allow to stand; the blue-red colour is gradually discharged.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -185$ to -193?

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution in ethanol. A solution of 1.0 g in 10 ml of ethanol (~750 g/l) TS is clear and not more intensely coloured than standard colour solution Yw3 or Rd1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 20 mg/g.

Related substances. Carry out the test as described below under "Assay".

Inject alternately 20 µl each of solutions A and C. Continue the recording of the chromatogram for 1.7 times the retention time of the principal peak.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than 0.7 times the area of the principal peak obtained with solution C (0.7%), and the sum of these areas is not greater than 1.5 times the area of the principal peak of the chromatogram obtained with solution C (1.5%).

Assay. Determine as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3–5 µm). The column is connected to the injection port by a steel capillary tube about 1 m long with an internal diameter of 0.25 mm. Maintain the temperature of the column and of the steel capillary at 80 °C. As the mobile phase, use a mixture of 52 volumes of water, 43 volumes of acetonitrile R, 5 volumes of *tert*-butyl methyl ether R, and 0.1 volume of phosphoric acid (~1440 g/l) TS.

Prepare the following solutions in a solvent mixture of equal volumes of acetonitrile R and water: solution (A) 1.2 mg of Ciclosporin per ml; solution (B) 1.2 mg of ciclosporin RS per ml; for solution (C) dilute 2.0 ml of solution B to 200 ml with the solvent mixture; and for solution (D) dissolve 3 mg of ciclosporin U RS in 2.5 ml of the solvent mixture and add 2.5 ml of solution B.

Operate with a flow rate of about 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject 20 µl of solution D. The assay is valid only if the relative standard deviation of the area of the principal peak is not more than 1.0%, unless the resolution between the two principal peaks is 1.0 and 1.8. The assay is not valid unless the retention time of the principal peak is between 25 and 30 minutes.

Inject alternately 20 µl each of solutions A and B.

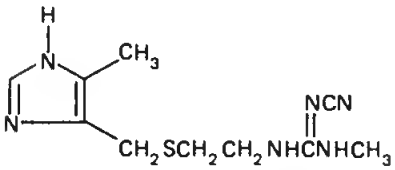
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{62}H_{111}N_{11}O_{12}$.

CIMETIDINUM
CIMETIDINE

Molecular formula. $C_{10}H_{16}N_6S$

Relative molecular mass. 252.3

Graphic formula.



Chemical name. 2-Cyano-1-methyl-3-[2-[[(5-methylimidazol-4-yl)methyl]-thio] ethyl]guanidine; *N*″-cyano-*N*-methyl-*N*′-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl] thio]ethyl]guanidine; 1-cyano-2-methyl-3-[2-[[(5-methylimidazol-4-yl)methyl] thio]ethyl]guanidine; *N*-cyano-*N*′-methyl-*N*″-[2-[[(5-methyl-1*H*-imidazol-4-yl) methyl]thio]ethyl]guanidine; CAS Reg. No. 51481-61-9.

Description. A white to off-white powder; odourless or with a faint odour.

Solubility. Sparingly soluble in water; very soluble in methanol R.

Category. Antiulcer drug.

Storage. Cimetidine should be kept in a well-closed container.

Additional information. Cimetidine exists in three polymorphic forms. The polymorph specified in the monograph corresponds to the crystal form of cimetidine RS.

Requirements

Definition. Cimetidine contains not less than 98.5% and not more than 101.0% of $C_{10}H_{16}N_6S$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum obtained from the solid state without prior solvent treatment is concordant with the spectrum similarly obtained from cimetidine RS or with the *reference spectrum* of cimetidine; no shoulder or peak is discernible at 1180 cm^{-1} (confirmation of polymorphic form).

B. Melting temperature, about 142 °C.

Heavy metals. Use 1.0 g for the preparation if the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10.0 mg/g.

pH value. pH of a 5.0 mg/ml solution in carbon-dioxide-free water R, 8.0–9.5.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column 25 cm long and 4.6 mm internal diameter, packed with particles of porous silica gel or ceramic, 5–10 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups. Prepare the following solvent mixture: Dilute 1 ml of glacial acetic acid R with sufficient water to produce 200 ml. To 190 ml of this solution add 10 ml of ammonium acetate (2 g/l) TS. As the mobile phase use a degassed and filtered mixture of 84 volumes of the above solvent mixture and 16 volumes of acetonitrile R. For the system suitability test prepare a solution containing 18 µg of cimetidine RS and 24 µg of caffeine RS per ml of the above solvent mixture (solution A). Further prepare a solution of the substance to be

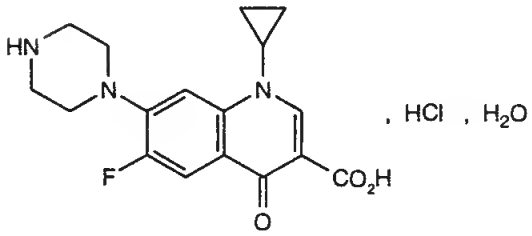
examined containing 18 µg per ml of solvent mixture (solution B). Operate with a flow rate of about 1 ml per minute. As detector use an ultraviolet spectrophotometer at a wavelength of about 228 nm, fitted with a suitable recorder. Make 6 replicate injections, each of 10 µl of solution A. Measure the peak responses; the relative standard deviation of the ratio of the responses from cimetidine to the sum of all the responses in the chromatogram, excluding any from the solvent mixture, is not more than 2.0%, and the resolution between caffeine and cimetidine is not less than 3.0. The relative retention times are about 1.0 for caffeine and 1.4 for cimetidine. Then inject 10 µl of solution B and measure the peak responses: the ratio of the response from cimetidine to the sum of all the responses in the chromatogram, excluding any from the solvent mixture, is not less than 0.99.

Assay. Dissolve about 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.23 mg of $C_{10}H_{16}N_6S$.

Additional requirement for Cimetidine for parenteral use

Complies with the monograph for "Parenteral preparations".

CIPROFLOXACINI HYDROCHLORIDUM
CIPROFLOXACIN HYDROCHLORIDE



$C_{17}H_{18}FN_3O_3,HCl,H_2O$

Relative molecular mass. 385.8

Chemical name. 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid monohydrochloride monohydrate; CAS Reg. No. 86393-32-0.

Description. A pale yellow, crystalline powder.

Solubility. Soluble in water; slightly soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and dichloromethane R.

Category. Antibacterial.

Storage. Ciprofloxacin hydrochloride should be kept in a tightly closed container, protected from light.

Requirements

Ciprofloxacin hydrochloride contains not less than **98.0%** and not more than **102.0%** of $C_{17}H_{18}FN_3O_3$,HCl, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ciprofloxacin hydrochloride RS or with the *reference spectrum* of ciprofloxacin hydrochloride.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 4 volumes of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate as 1-cm bands, 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin hydrochloride per ml, and (B) 10 mg of ciprofloxacin hydrochloride RS per ml. Place an evaporating-dish containing 50 ml of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm and 365 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. A 0.1 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. For the preparation of the test solution dissolve 0.25 g in water and dilute to 30 ml with the same solvent. Carry out the prefiltration. Determine the heavy metals content in the filtrate as described under 2.2.3 Limit test for heavy metals, Method B; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.25 g in 10 ml of carbondioxide-free water R is clear and not more intensely coloured than standard colour solution Gn4 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method B, using about 0.2 g of the substance; the water content is between 0.047 g/g and 0.067 g/g.

pH value. pH of a 25 mg/ml solution in carbon-dioxide-free water R, 3.0–4.5.

Fluoroquinolonic acid. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 4 volumes of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin hydrochloride per ml, and for solution (B) dissolve 10 mg of fluoroquinolonic acid RS in a mixture of 0.10 ml of ammonia (~100 g/l) TS and 90 ml of water, and dilute to 100 ml with water. Dilute 2.0 ml of this solution to 10 ml with water. Place an evaporating-dish containing 50 ml of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

The spot corresponding to fluoroquinolonic acid in the chromatogram obtained with solution A is not more intense than that obtained with solution B (0.2%).

Related substances. Carry out the test as described below under "Assay".

Inject 50 µl each of solutions A and F. Record the chromatogram for twice the retention time of ciprofloxacin.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and F, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the areas of the peaks corresponding to the ethylenediamine compound and the by-compound A are not greater than the corresponding peaks in the chromatogram obtained with solution F (0.2%); the area of any other peak is not greater than the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained with solution F (0.2%); the sum of the areas of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained

with solution F (0.5%). Disregard any peak with an area less than 0.25 times the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained with solution F (0.05%).

Assay. Determine as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 87 volumes of phosphoric acid (~2.45 g/l) TS, adjusted to a pH of 3.0 with triethylamine R and 13 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase to produce 50 ml: solution (A) contains 0.50 mg of Ciprofloxacin hydrochloride per ml; solution (B) contains 0.50 mg of ciprofloxacin hydrochloride RS per ml; solution (C) contains 0.050 mg of 1-cyclopropyl-1,4-dihydro-4-oxo-7-(1-piperazin-1-yl)quinoline-3-carboxylic acid RS per ml (desfluoro compound); solution (D) contains 0.050 mg of 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid RS (ethylenediamine compound) per ml; solution (E) contains 0.050 mg of 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS (by-compound A) per ml. For solution (F) mix 0.1 ml of solution A with 1.0 ml of solution C, 1.0 ml of solution D, 1.0 ml of solution E, and dilute to 50 ml with the mobile phase.

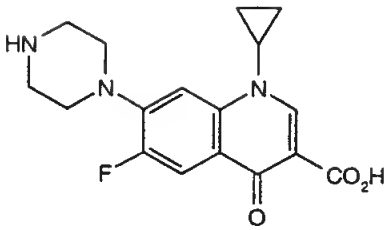
Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 278 nm. Maintain the temperature of the column at 40 °C.

Inject 50 µl of solution F. The following order of elution is obtained: desfluoro compound, ethylenediamine compound, ciprofloxacin and by-compound A. The retention time of ciprofloxacin is about 9 minutes. Adjust the sensitivity of the system so that the height of the peak due to the ethylenediamine compound is at least 40% of the full scale of the recorder. The assay is not valid unless the resolution between the peaks corresponding to the desfluoro compound and the ethylenediamine compound is at least 1.3, and the resolution between the peaks corresponding to ciprofloxacin and the by-compound A is at least 3.0. Inject 10 µl of solution B. The assay is not valid unless the relative standard deviation of the peak area of ciprofloxacin is at most 1.0%.

Inject alternately 10 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{17}H_{18}FN_3O_3$,HCl.

CIPROFLOXACINUM
CIPROFLOXACIN



$C_{17}H_{18}FN_3O_3$

Relative molecular mass. 331.4

Chemical name. 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid; CAS Reg. No. 85721-33-1.

Description. A white to pale yellow, crystalline powder.

Solubility. Practically insoluble in water; very slightly soluble in ethanol (~750 g/l) TS and dichloromethane R.

Category. Antibacterial.

Storage. Ciprofloxacin should be kept in a well-closed container, protected from light.

Additional information. Ciprofloxacin exists in different polymorphic forms.

Requirements

Ciprofloxacin contains not less than **98.0%** and not more than **102.0%** of $C_{17}H_{18}FN_3O_3$, calculated with reference to the dried substance.

Identity test
Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ciprofloxacin RS or with the *reference spectrum* of ciprofloxacin.

Heavy metals. For the preparation of the test solution dissolve 0.5 g in acetic acid (~60 g/l) TS and dilute to 30 ml with the same solvent. Carry out the prefiltration. To the filtrate add 2.0 ml of water and determine the heavy metals

content as described under 2.2.3 Limit test for heavy metals, Method B; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.25 g in 20 ml of hydrochloric acid (0.1 mol/l) VS is clear and not more intensely coloured than standard colour solution Gn4 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 120 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury); it loses not more than 10 mg/g.

Fluoroquinolonic acid. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 4 volumes of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin per ml of acetic acid (~60 g/l) TS, and for solution (B) dissolve 10 mg of fluoroquinolonic acid RS in a mixture of 0.10 ml of ammonia (~100 g/l) TS and 90 ml of water, and dilute to 100 ml with water. Dilute 2.0 ml of this solution to 10 ml with water. Place an evaporating-dish containing 50 ml of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

The spot corresponding to fluoroquinolonic acid in the chromatogram obtained with solution A is not more intense than that obtained with solution B (0.2%).

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography (5 µm). As the mobile phase, use a mixture of 87 volumes of phosphoric acid (~2.8 g/l) TS adjusted to a pH of 3.0 with triethylamine R and 13 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (A) add 0.2 ml of phosphoric acid (~105 g/l) TS to 25 mg of Ciprofloxacin, dilute to 50 ml, and treat in an ultrasonic bath until a clear solution is obtained. For solution (B) dilute 0.10 ml of solution A to 50 ml. For solution (C) use 2.5 mg of 1-cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid RS (desfluoro compound) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (D) use 2.5 mg of 7-[(2-aminoethyl)amino]-1-

cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid RS (ethylenediamine compound) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (E) use 2.5 mg of 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS (by-compound A) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (F) mix 0.1 ml of solution A with 1.0 ml of each of solutions C, D, and E, prior to dilution as described above, and dilute to 50 ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 278 nm. Maintain the temperature of the column at 40 °C.

Inject alternately 50 µl each of solutions B, C, D, E, and F. The retention time of ciprofloxacin is about 9 minutes. Adjust the sensitivity of the system so that the height of the peak due to the ethylenediamine compound obtained with solution F is at least 40% of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to the desfluoro compound and the ethylenediamine compound in the chromatogram obtained with solution F is at least 1.3, and the resolution between the peaks corresponding to ciprofloxacin and the by-compound A is at least 3.0.

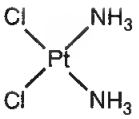
Inject alternately 50 µl each of solutions A, D, and E. Record the chromatogram for twice the retention time of ciprofloxacin.

Measure the areas of the peak responses obtained in the chromatograms from solutions A, D, and E, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the areas of the peaks corresponding to the ethylenediamine compound and by-compound A are not greater than the corresponding peaks obtained with solutions D and E (0.2%). The area of any other peak is not greater than the area of the peak obtained with solution D (0.2%). The sum of the areas of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution D (0.5%). Disregard any peak with an area less than 0.25 times the area of the peak obtained with solution D (0.05%).

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.14 mg of $C_{17}H_{18}FN_3O_3$.

CISPLATINUM
CISPLATIN



$Cl_2H_6N_2Pt$

Relative molecular mass. 300.0

Chemical name. *cis*-Diamminedichloroplatinum; (*SP*-4-2)-diamminedichloroplatinum; CAS Reg. No. 15663-27-1.

Description. White to yellowish crystals or a yellow powder.

Solubility. Slightly soluble in water; sparingly soluble in dimethylformamide R; practically insoluble in methanol R.

Category. Cytotoxic drug.

Storage. Cisplatin should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Additional information. *CAUTION:* Cisplatin must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

When heated, it blackens at about 270 °C with decomposition.

Requirements

Cisplatin contains not less than **96.0%** and not more than the equivalent of **102.0%** of $Cl_2H_6N_2Pt$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from cisplatin RS or with the *reference spectrum* of cisplatin.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Place 0.05 g in a glass dish, add 2 ml of sodium hydroxide (~80 g/l) TS, and evaporate to dryness on a water-bath. Dissolve the residue in a mixture of 0.5 ml of nitric acid (~1000 g/l) TS and 1.5 ml of hydrochloric acid (~420 g/l) TS, and again evaporate to dryness; the residue is orange. Again dissolve the residue in 0.5 ml of water and add 0.5 ml of ammonium chloride (100 g/l) TS; a yellow, crystalline precipitate is produced.

Clarity and colour of solution. Dissolve 25 mg in 25 ml of a solution of 0.22 g of sodium chloride R dissolved in 25 ml of carbon-dioxide-free water R; the solution is clear and not more intensely coloured than standard colour Gn3 when compared as described under 1.11 Colour of liquids. (Keep the solution for the "pH value".)

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g of Cisplatin; the water content is not more than 10 mg/g.

pH value. Measure without delay the pH of the solution prepared for the "Clarity and colour of solution"; 4.5–6.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R2 previously activated by heating at 150 °C as the coating substance and a mixture of 1 volume of water and 9 volumes of acetone R as the mobile phase. Apply separately to the plate 2.5 µl of each of two solutions in a mixture of equal volumes of dimethylformamide R and water containing (A) 2 mg of Cisplatin per ml and (B) 2 mg of cisplatin RS per ml. Also apply 5 µl of each of two solutions in dimethylformamide R containing (C) 20 mg of Cisplatin per ml and (D) 0.4 mg of Cisplatin per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, spray it with stannous chloride/ hydrochloric acid TS, and allow to dry again. Examine the chromatogram in daylight.

With solution C no spot occurs in front of the principal spot. Any other spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D.

Ultraviolet absorbance ratio. Prior to use, clean all glassware with a mixture of 3 volumes of hydrochloric acid (~420 g/l) TS and 1 volume of nitric acid (~1000 g/l) TS, rinse thoroughly with water, and dry. Do not use any dichromate solution for cleaning, or acetone or pressurized air for drying. Protect the test solutions from light, and use them within 1 hour of preparation.

Transfer about 98.5 mg, accurately weighed, to a 100-ml volumetric flask and dissolve in sufficient hydrochloric acid (0.1 mol/l) VS to produce 100 ml. Stir with a magnetic bar at a high speed for 5 minutes or place in an ultrasonic bath for 10 seconds or until completely dissolved.

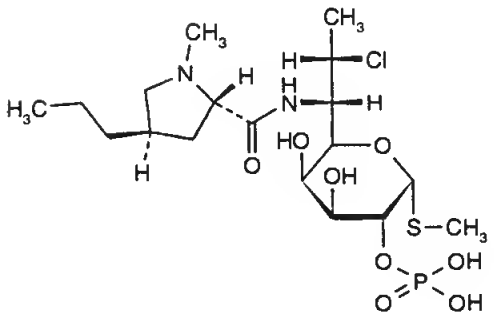
The ratio of the absorbance measured in a 1-cm layer against hydrochloric acid (0.1 mol/l) VS at the maximum wavelength of about 301 nm to that at the minimum wavelength of about 246 nm is not less than 4.5.

Silver. Determine by atomic absorption spectrophotometry "1.8 Atomic spectrometry: emission and absorption" at a wavelength of 328 nm using a silver hollow cathode lamp, an air-acetylene flame, and a slit width of 0.5 nm. Dissolve 0.1 g in 15 ml of nitric acid (~1000 g/l) TS while heating at 80 °C and dilute with water to 25 ml. As a reference solution use silver standard (5 µg Ag/ml) TS; not more than 250 µg of Ag per g.

Assay. Dissolve about 25 mg, accurately weighed, in sufficient hydrochloric acid (~70 g/l) TS to produce 25 ml. Dilute 1.0 ml of this solution with the same acid to 25 ml. Transfer 5 ml to a glass-stoppered 25-ml volumetric flask and add 10 ml of hydrochloric acid (~70 g/l) TS. Place 15 ml of hydrochloric acid (~70 g/l) TS in a second flask to serve as a blank. Add 2.5 ml of stannous chloride/hydrochloric acid TS1 and dilute to volume with hydrochloric acid (~70 g/l) TS. Mix and allow to stand for 30 minutes.

Measure the absorbance of a 1-cm layer at the maximum at about 402 nm against a solvent cell containing the blank. Calculate the amount of $Cl_2H_6N_2Pt$ in Cisplatin being examined by comparison with cisplatin RS, similarly and concurrently examined.

CLINDAMYCINI PHOSPHAS
CLINDAMYCIN PHOSPHATE



$C_{18}H_{34}ClN_2O_8PS$

Relative molecular mass. 505.0

Chemical name. (2*S*-trans)-Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-(dihydrogen phosphate); CAS Reg. No. 24729-96-2.

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antibacterial drug.

Storage. Clindamycin phosphate should be kept in a tightly closed container.

Labelling. The designation Clindamycin phosphate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. Clindamycin phosphate is slightly hygroscopic.

Requirements

Clindamycin phosphate contains not less than **95.0%** and not more than **100.5%** of $C_{18}H_{34}ClN_2O_8PS$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from clindamycin phosphate RS or with the *reference spectrum* of clindamycin phosphate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 6 volumes of 1-butanol R, 2 volumes of water, and 2 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 2.0 mg of Clindamycin phosphate per ml, (B) 2.0 mg of clindamycin phosphate RS, and for solution (C) dissolve 10 mg of lincomycin hydrochloride RS in 5 ml of solution B. After removing the plate from the chromatographic chamber, allow it to dry at 105 °C for 30 minutes, and spray with potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

C. Dissolve 10 mg in 2 ml of hydrochloric acid (~70 g/l) TS and heat directly in a flame for 1 minute; a disagreeable sulfurous odour is perceptible. Cool, add 4 ml of sodium carbonate (75 g/l) TS and 0.5 ml of sodium nitroprusside (45 g/l) TS; a violet-red ring is formed that fades quickly.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 ml of sodium hydroxide (~400 g/l) TS and 5 ml of water for 90 minutes. Cool and add 5 ml of nitric acid (~1000 g/l) TS. Extract with three 15-ml quantities of dichloromethane R, and discard the extracts. Filter the aqueous layer through a paper filter; the filtrate yields reaction B described under 2.1 General identification tests as characteristic of orthophosphates.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +115°$ to $+130°$.

Clarity and colour of solution. A solution of 0.040 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g of the substance; the water content is not more than 0.060 g/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 3.5–4.5.

Related substances. Carry out the test as described below under "Assay".

Inject alternately 20 µl each of solutions A and D. Continue the recording of the chromatogram until clindamycin is eluted.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and D, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak or any peak corresponding to the solvent, is not greater than 2.5 times the area of the principal peak obtained with solution D (2.5%). The sum of the areas of all the peaks, other than the principal peak or any peak corresponding to the solvent, is not greater than 4 times the peak corresponding to clindamycin phosphate obtained with solution D (4.0%).

Assay. Determine as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically

bonded octadecylsilyl groups (5–10 µm). As the mobile phase, use a mixture of 8 volumes of potassium dihydrogen phosphate (13.6 g/l) TS adjusted to pH 2.5 with phosphoric acid (~105 g/l) TS and 2 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase: solution (A) 3.0 mg of Clindamycin phosphate per ml; solution (B) 3.0 mg of clindamycin phosphate RS per ml; for solution (C) dissolve 5 mg of lincomycin hydrochloride RS and 15.0 mg of clindamycin hydrochloride RS in 5.0 ml of solution B and dilute with sufficient mobile phase to produce 100 ml; and for solution (D) dilute 1.0 ml of solution B with sufficient mobile phase to produce 100 ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject 20 µl of solution C.

The assay is not valid unless the first peak (lincomycin) is clearly separated from the solvent peak, and the resolution between the second peak (clindamycin phosphate) and the third peak (clindamycin) is at least 6.0. The assay is valid only if the symmetry factor of the clindamycin phosphate peak is not greater than 1.5.

Inject 20 µl of solution B. If necessary adjust the integrator parameters.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained with solutions A and B, and calculate the percentage content of $C_{18}H_{34}ClN_2O_8PS$.

Additional requirements for Clindamycin phosphate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.6 IU of endotoxin RS per mg of clindamycin.

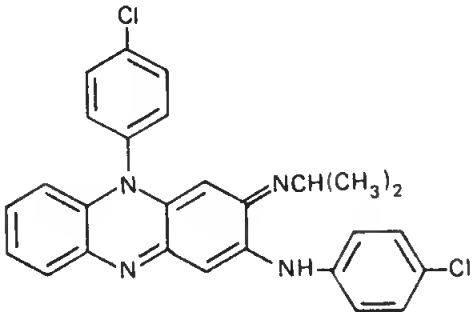
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using a solution in water containing 150 mg of Clindamycin phosphate per ml.

CLOFAZIMINUM
CLOFAZIMINE

Molecular formula. $C_{27}H_{22}Cl_2N_4$

Relative molecular mass. 473.4

Graphic formula.



Chemical name. 3-(*p*-Chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine; *N*,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl) imino]-2-phenazinamine; CAS Reg. No. 2030-63-9.

Description. A reddish brown, fine powder; odourless or almost odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and in ether R.

Category. Antileprosy drug.

Storage. Clofazimine should be kept in a well-closed container.

Additional information. Clofazimine melts at about 217 °C.

Requirements

Definition. Clofazimine contains not less than 98.0% and not more than 101.0% of $C_{27}H_{22}Cl_2N_4$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the

spectrum obtained from clofazimine RS or with the *reference spectrum* of clofazimine.

B. The absorption spectrum of a 5.0 µg/ml solution in hydrochloric acid/methanol (0.01 mol/l) VS, when observed between 230 nm and 600 nm, exhibits 2 maxima at about 283 nm and 487 nm. The absorbances of a 1-cm layer at these wavelengths are about 0.65 and 0.32, respectively.

C. Dissolve about 2 mg in 3 ml of acetone R and add 0.1 ml of hydrochloric acid (~420 g/l) TS; an intense violet colour is produced. Add 0.5 ml of sodium hydroxide (~200 g/l) TS; the colour changes to orange-red.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 (a precoated plate from a commercial source is suitable), exposed immediately before use to ammonia vapour by suspending the plate for 30 minutes in a chromatographic chamber containing a shallow layer of ammonia (~17 g/l) TS. As the mobile phase, to be used in a separate chamber, prepare a mixture of 85 volumes of dichloromethane R and 4 volumes of 1-propanol R. Apply separately to the plate 5 µl of each of 3 solutions in chloroform R containing (A) 20 mg of the test substance per ml, (B) 0.16 mg of the test substance per ml, and (C) 0.10 mg of the test substance per ml. Allow the mobile phase to ascend 12 cm above the line of application. After removing the plate from the chromatographic chamber, allow it to dry in air for 5 minutes, and replace it in the chamber. Allow the mobile phase to ascend again 12 cm, remove the plate from the chamber, dry it in air for 5 minutes, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than the spot obtained with solution C, except that 2 such spots are not more intense than that obtained with solution B.

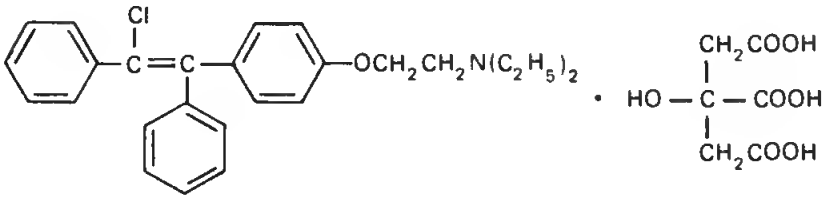
Assay. Dissolve about 0.4 g, accurately weighed, in 20 ml of chloroform R, add 50 ml of acetone R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 47.34 mg of $C_{27}H_{22}Cl_2N_4$.

CLOMIFENI CITRAS
CLOMIFENE CITRATE

Molecular formula. $C_{26}H_{28}ClNO,C_6H_8O_7$

Relative molecular mass. 598.1

Graphic formula.



Chemical name. 2-[*p*-(2-Chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate (1:1); 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-*N,N*-diethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1); CAS Reg. No. 50-41-9.

Description. A white to pale yellow powder; odourless.

Solubility. Slightly soluble in water; freely soluble in methanol R; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Ovulation inducer.

Storage. Clomifene citrate should be kept in a well-closed container, protected from light.

Additional information. Clomifene citrate is a mixture of the *E* and *Z* geometric isomers.

Requirements

Definition. Clomifene citrate contains not less than 97.0% and not more than 101.0% of $C_{26}H_{28}ClNO,C_6H_8O_7$, and not less than 30.0% and not more than 50.0% of the *Z*-isomer, both calculated with reference to the anhydrous substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from clomifene citrate RS or with the *reference spectrum* of clomifene citrate.

B. The ultraviolet absorption spectrum of a 25 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 220 nm and 350 nm, exhibits maxima at about 235 nm and 292 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.79 and 0.44, respectively.

C. A 10 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of citrates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Solution in methanol. A solution of 1.0 g in 30 ml of methanol R is clear and colourless.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 10 mg/g.

***Z*-isomer.** Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 90 volumes of chloroform R, 10 volumes of methanol R, and 1 volume of water as the mobile phase. Dissolve 100 mg of the test substance in 50 ml of a mixture of 3 volumes of chloroform R and 1 volume of ethanol (~750 g/l) TS (solution A), and dissolve 50 mg of clomifene citrate *Z*-isomer RS in 50 ml of the same solvent mixture (solution B). With a syringe apply 100 µl of solution A to one side and 100 µl of solution B to the other side, keeping the centre of the plate to serve as a blank. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Mark the bands of the *Z*-isomer with a pencil, scratch off separately the bands of silica gel produced by solutions A and B, as well as a band of a similar size from the centre of the plate for the blank, and transfer them to separate test-tubes. Add 10 ml of ethanol (~750 g/l) TS to each tube, shake vigorously and then centrifuge or filter. Measure the absorbances of a 1-cm layer of the filtered solutions at 240 nm against a solvent cell containing ethanol (~750 g/l) TS. Calculate the content of *Z*-isomer in the following manner: Deduct the absorbance of the blank solution from the absorbances of the test and reference solutions and apply the formula: $(A_1)\ (W_2)\ (1000)/(A_2)\ (W_1)$, where A_1 is the absorbance of the test substance, A_2 the absorbance of clomifene citrate *Z*-isomer RS, W_1 the weight in mg of the test substance, and W_2 the weight in

mg of clomifene citrate *Z*-isomer RS; the content of the *Z*-isomer is not less than 300 mg/g and not more than 500 mg/g.

Assay. Dissolve about 1.0 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 59.81 mg of $C_{26}H_{28}ClNO,C_6H_8O_7$.

CLOXACILLINUM NATRICUM

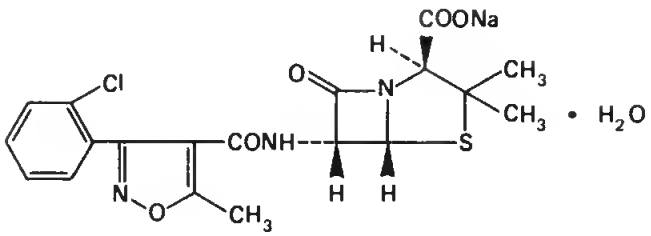
CLOXACILLIN SODIUM

Cloxacillin sodium (non-injectable)
Cloxacillin sodium, sterile

Molecular formula. $C_{19}H_{17}ClN_3NaO_5S,H_2O$

Relative molecular mass. 475.9

Graphic formula.



Chemical name. Sodium (2*S*,5*R*,6*R*)-6-[3-(*o*-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate; sodium [2*S*-(2α,5α,6β)]-6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylate monohydrate; monosodium [3-(*o*-chlorophenyl)-5-methyl-4-isoxazolyl]penicillin monohydrate; CAS Reg. No. 7081-44-9.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 2.5 parts of water and in 30 parts of ethanol (~750 g/l) TS.

Category. Antibiotic.

Storage. Cloxacillin sodium should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Cloxacillin sodium indicates that the substance complies with the additional requirements for sterile Cloxacillin sodium and may be used for parenteral administration or for other sterile applications.

Additional information. Cloxacillin sodium is hygroscopic. Even in the absence of light, Cloxacillin sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Cloxacillin sodium contains not less than 90.0% of total penicillins calculated as $C_{19}H_{18}ClN_3O_5S$, and with reference to the anhydrous substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from cloxacillin sodium RS or with the *reference spectrum* of cloxacillin sodium.

B. Place 2 mg into a test-tube, add 2 mg of disodium chromotropate R and 2 ml of sulfuric acid (~1760 g/l) TS. Immerse the tube in a suitable bath at 150 °C for 3–4 minutes; a purple colour is produced.

C. Ignite 20 mg and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a 10 mg/ml solution, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +163 to +172 °.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not less than 35 mg/g and not more than 45 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 5.0–7.0.

Chlorine. Carry out the combustion as described under the 2.4 Oxygen flask method, but using 0.040 g of the test substance and 20 ml of sodium hydroxide (1 mol/l) VS as the absorbing liquid. When the process is complete, add 2.5 ml of nitric acid (~130 g/l) TS, 2.5 ml of water, and 20 ml of silver nitrate

(0.01 mol/l) VS and titrate with ammonium thiocyanate (0.01 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the operation without the substance being tested. Each ml of silver nitrate (0.01 ml/l) VS is equivalent to 0.3546 mg of Cl; the chlorine content is 70–75 mg/g.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A).

To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 343 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{19}H_{17}ClN_3NaO_5S$ in the substance being tested by comparison with cloxacillin sodium RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.40 ± 0.02.

Additional requirements for Cloxacillin Sodium for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 40 IU of endotoxin RS per mg of cloxacillin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

CODEINI PHOSPHAS

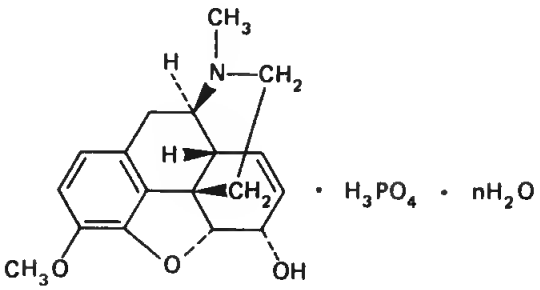
CODEINE PHOSPHATE

Codeine phosphate hemihydrate
Codeine phosphate sesquihydrate

Molecular formula. $C_{18}H_{21}NO_3$, H_3PO_4, $\frac{1}{2}H_2O$ (hemihydrate); $C_{18}H_{21}NO_3$, H_3PO_4, $1\frac{1}{2}H_2O$ (sesquihydrate).

Relative molecular mass. 406.4 (hemihydrate); 424.4 (sesquihydrate).

Graphic formula.



n = $\frac{1}{2}$ (hemihydrate)
n = $1\frac{1}{2}$ (sesquihydrate)

Chemical name. 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate (1 : 1) (salt) hemihydrate; CAS Reg. No. 41444-62-6 (hemihydrate). 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol phosphate (1 : 1) (salt) sesquihydrate; CAS Reg. No. 5913-76-8 (sesquihydrate).

Description. Small, colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 4 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antitussive; analgesic.

Storage. Codeine phosphate should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container should state if the Codeine phosphate is the hemihydrate or the sesquihydrate.

Additional information. Codeine phosphate effloresces in dry air.

Requirements

Definition. Codeine phosphate contains not less than 98.0% and not more than 101.0% of $C_{18}H_{21}NO_3,H_3PO_4$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 1 ml of sulfuric acid (~1760 g/l) TS, add 1 drop of ferric chloride (25 g/l) TS, and warm on a water-bath; a blue colour is produced, which changes to red on the addition of 1 drop of nitric acid (~130 g/l) TS.

B. Dissolve 1 mg in 0.5 ml of selenious acid/sulfuric acid TS; a green colour is produced, which rapidly changes to blue, then slowly to dark yellow-green.

C. Neutralize a 20 mg/ml solution with ammonia (~100 g/l) TS; it yields reaction B described under 2.1 General identification tests as characteristic of orthophosphates.

D. To 5 ml of a 0.2 g/ml solution add 1 ml of ammonia (~100 g/l) TS, cool and scratch the inside of the test-tube to induce crystallization. Wash the precipitate with ethanol (~750 g/l) TS and dry at 105 °C. Melting temperature, about 156 °C (codeine base).

Specific optical rotation. Use a 20 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -98$ to $-102°$.

Chlorides. Dissolve 0.70 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.35 mg/g.

Sulfates. Dissolve 0.50 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Clarity and colour of solution. A solution of 0.40 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C: Codeine phosphate (hemihydrate) loses not more than 30 mg/g. Codeine phosphate sesquihydrate loses not less than 50 mg/g and not more than 70 mg/g.

pH value. pH of a 0.04 g/ml solution, 4.2–5.0.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 72 volumes of ethanol (~750 g/l) TS, 30 volumes of cyclohexane R and 6 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 4 volumes of hydrochloric acid (0.01 mol/l) VS and 1 volume of ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per ml and (B) 0.66 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in

daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

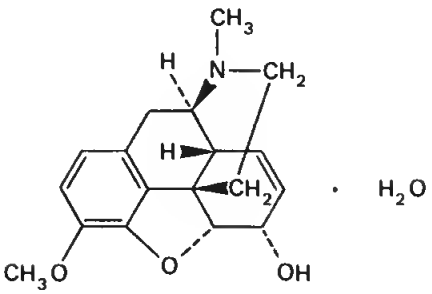
Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 39.74 mg of $C_{18}H_{21}NO_3,H_3PO_4$.

CODEINUM MONOHYDRICUM
CODEINE MONOHYDRATE

Molecular formula. $C_{18}H_{21}NO_3,H_2O$

Relative molecular mass. 317.4

Graphic formula.



Chemical name. 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol monohydrate; CAS Reg. No. 6059-47-8.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Antitussive; analgesic.

Storage. Codeine monohydrate should be kept in a tightly closed container, protected from light.

Additional information. Codeine monohydrate effloresces slowly in dry air.

Requirements

Definition. Codeine monohydrate contains not less than 99.0% and not more than 101.0% of $C_{18}H_{21}NO_3$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 1 ml of sulfuric acid (~1760 g/l) TS, add 1 drop of ferric chloride (25 g/l) TS, and warm on a water-bath; a blue colour is produced, which changes to red on the addition of 1 drop of nitric acid (~130 g/l) TS.

B. Dissolve 1 mg in 0.5 ml of selenious acid/sulfuric acid TS; a green colour is produced, which rapidly changes to blue, then slowly to dark yellow-green.

C. Melting temperature, about 156 °C.

Specific optical rotation. Use a 20 mg/ml solution in ethanol (~750 g/l) TS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -142$ to $-146°$.

Clarity and colour of solution. A solution of 0.050 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 50 mg/g and not more than 60 mg/g.

pH value. pH of a 5.0 mg/ml solution in carbon-dioxide-free water R, not less than 9.0.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 72 volumes of ethanol (~750 g/l) TS, 30 volumes of cyclohexane R, and 6 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 4 volumes of hydrochloric acid (0.01 mol/l) VS and 1 volume of ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per ml and (B) 0.66 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 29.94 mg of $C_{18}H_{21}NO_3$.

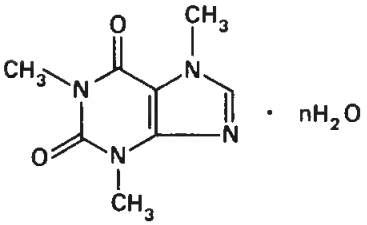
COFFEINUM
CAFFEINE

Caffeine anhydrous
Caffeine monohydrate

Molecular formula. $C_8H_{10}N_4O_2$ (anhydrous); $C_8H_{10}N_4O_2,H_2O$ (monohydrate).

Relative molecular mass. 194.2 (anhydrous); 212.2 (monohydrate).

Graphic formula.



n = 0 (anhydrous)
n = 1 (monohydrate)

Chemical name. 3,7-Dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione; CAS Reg. No. 58-08-2 (anhydrous). 3,7-Dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione monohydrate; CAS Reg. No. 5743-12-4 (monohydrate).

Description. Silky, colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 60 parts of water and in 100 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Central nervous stimulant.

Storage. Caffeine should be kept in a well-closed container.

Labelling. The designation on the container of Caffeine should state whether the substance is the monohydrate or is in the anhydrous form.

Additional information. Caffeine monohydrate is efflorescent in air.

Requirements

Definition. Caffeine contains not less than 98.5% and not more than 101.0% of $C_8H_{10}N_4O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or all 3 tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For Caffeine monohydrate, the substance must be previously dried to constant weight at 80 °C. The infrared absorption spectrum is concordant with the spectrum obtained from caffeine RS or with the *reference spectrum* of caffeine.

B. To 10 mg, contained in a porcelain dish, add 1 ml of hydrochloric acid (~250 g/l) TS and 0.5 ml of hydrogen peroxide (~60 g/l) TS, and evaporate to dryness on a water-bath. Add 1 drop of ammonia (~100 g/l) TS; the residue acquires a purple colour, which disappears upon the addition of 2–3 drops of sodium hydroxide (~80 g/l) TS.

C. To a saturated solution add a few drops of iodine TS; the solution remains clear. Add a few drops of hydrochloric acid (~70 g/l) TS; a brown precipitate is produced. On neutralization with sodium hydroxide (~80 g/l) TS, the precipitate dissolves.

D. Melting temperature, after drying at 80 °C, about 236 °C.

Clarity and colour of solution. A solution of 5.0 g in 10 ml of boiling water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 80 °C: Caffeine (anhydrous) loses not more than 5.0 mg/g. Caffeine monohydrate loses not less than 50 mg/g and not more than 90 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 4.8–6.6.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 4 volumes of 1-butanol R, 3 volumes of chloroform R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Prepare 2 solutions in a mixture of 6 volumes of chloroform R and 4 volumes of methanol R containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. Apply separately to the plate 10 µl of solution A and 5 µl of solution B. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

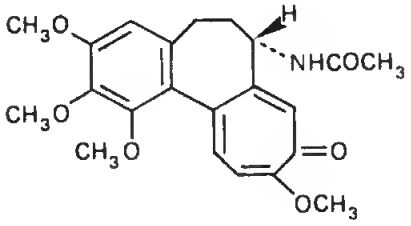
Assay. Dissolve about 0.18 g, accurately weighed, in 10 ml of acetic anhydride R and 20 ml of toluene R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 19.42 mg of $C_8H_{10}N_4O_2$.

COLCHICINUM
COLCHICINE

Molecular formula. $C_{22}H_{25}NO_6$

Relative molecular mass. 399.4

Graphic formula.



Chemical name. (*S*)-*N*-(5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo [*a*]heptalen-7-yl)acetamide; CAS Reg. No. 64-86-8.

Description. Pale yellow to pale greenish yellow crystals, amorphous scales or a powder; odourless or almost odourless.

Solubility. Soluble in water; freely soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Antigout drug.

Storage. Colchicine should be kept in a tightly closed container, protected from light.

Additional information. Colchicine is an alkaloid obtained from *Colchicum autumnale L.* (Fam. Liliaceae). It darkens on exposure to light. CAUTION: Colchicine is extremely poisonous and must be handled with care.

Requirements

Definition. Colchicine contains not less than 97.0% and not more than 103.0% of $C_{22}H_{25}NO_6$, calculated with reference to the anhydrous and solvent-free substance.

Identity tests

- Either test A or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from colchicine RS or with the *reference spectrum* of colchicine.

B. The absorption spectrum of a 10 µg/ml solution in ethanol (~750 g/l) TS, when observed between 230 nm and 400 nm, exhibits 2 maxima at about 243 nm and 350 nm.

C. Dissolve 30 mg in 1 ml of ethanol (~750 g/l) TS and add 1 drop of ferric chloride (25 g/l) TS; a red colour is immediately produced.

D. Mix 1 mg with about 0.2 ml of sulfuric acid (~1760 g/l) TS; a lemon-yellow colour is produced. Add about 0.1 ml of nitric acid (~1000 g/l) TS; the colour changes to greenish blue, rapidly becoming reddish and finally yellow or almost colourless. Then add a few drops of sodium hydroxide (~80 g/l) TS; the colour changes to red.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried and solvent-free substance; $[\alpha]_D^{20\,°C} = -425$ to $-460\,°$.

Sulfated ash. Not more than 1.0 mg/g.

Content of solvent and water. Dry at 130 °C for 4 hours, using about 0.5 g of the substance, and determine the loss of weight. Weigh 0.3 g of the dried material and determine the water content as described under 2.8 Determination of water by the Karl Fischer method, Method A, using pyridine R as the solvent; the sum of the loss of weight and of the water content, both expressed in mg/g, is not less than 115 mg/g and not more than 145 mg/g.

Colchiceine. Dissolve 0.050 g in 4 ml of water, add 0.2 ml of ferric chloride (25 g/l) TS, dilute to 6 ml with water, and mix; the colour produced in the test solution is not more intense than that of the standard colour solution produced by mixing 2 ml of iron colour TS, 1 ml of cobalt colour TS, 2 ml of copper colour TS and 0.70 ml of hydrochloric acid (~70 g/l) TS, when compared as described under 1.11 Colour of liquids.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a suitable aluminium oxide containing a substance that fluoresces at about 254 nm as the coating substance and a mixture of 25 volumes of chloroform R, 20 volumes of acetone R, and 0.4 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per ml and (B) 2.5 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

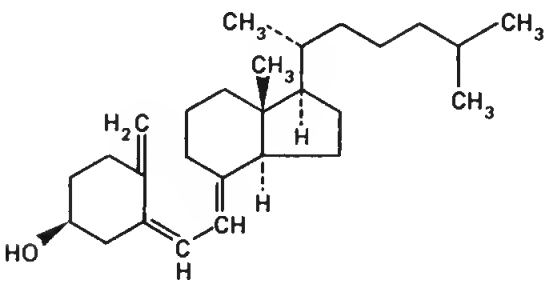
Assay. Dissolve about 0.05 g, accurately weighed, in a mixture of 10 ml of acetic anhydride R and 20 ml of toluene R, and titrate with perchloric acid (0.02 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.02 mol/l) VS is equivalent to 7.988 mg of $C_{22}H_{25}NO_6$.

COLECALCIFEROLUM
COLECALCIFEROL

Molecular formula. $C_{27}H_{44}O$

Relative molecular mass. 384.7

Graphic formula.



Chemical name. (5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β-ol; CAS Reg. No. 67-97-0.

Other name. Cholecalciferol.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS, ether R.

Category. Vitamin, antirachitic.

Storage. Colecalciferol should be kept in a hermetically closed container, in an inert atmosphere, protected from light and stored in a cool place.

Additional information. Even in the absence of light, Colecalciferol is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Colecalciferol contains not less than 95.0% and not more than 105.0% of $C_{27}H_{44}O$.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from colecalciferol RS or with the *reference spectrum* of colecalciferol.

B. Dissolve 1 mg in 1 ml of dichloroethane R and add 4 ml of antimony trichloride TS; a yellowish orange colour is produced.

C. Dissolve 5 mg in 5 ml of chloroform R, add 0.3 ml of acetic anhydride R and 0.1 ml of sulfuric acid (~1760 g/l) TS, and shake vigorously; a bright red colour is produced which changes rapidly through violet to blue and finally to green.

Specific optical rotation. Use a freshly prepared 10 mg/ml solution in aldehyde-free ethanol (~750 g/l) TS; $[\alpha]_D^{20\,°C} = +105$ to $+112°$.

7-Dehydrocholesterol. Dissolve 0.04 g in 2 ml of ethanol (~750 g/l) TS, using a glass-stoppered test-tube, and add 1 ml of digitonin TS; the solution produced remains clear for 12 hours.

Assay. With the aid of heat, dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure without delay the absorbance of a 1-cm layer of the diluted solution at the maximum at about 265 nm. Calculate the amount of $C_{27}H_{44}O$ in the substance being examined by

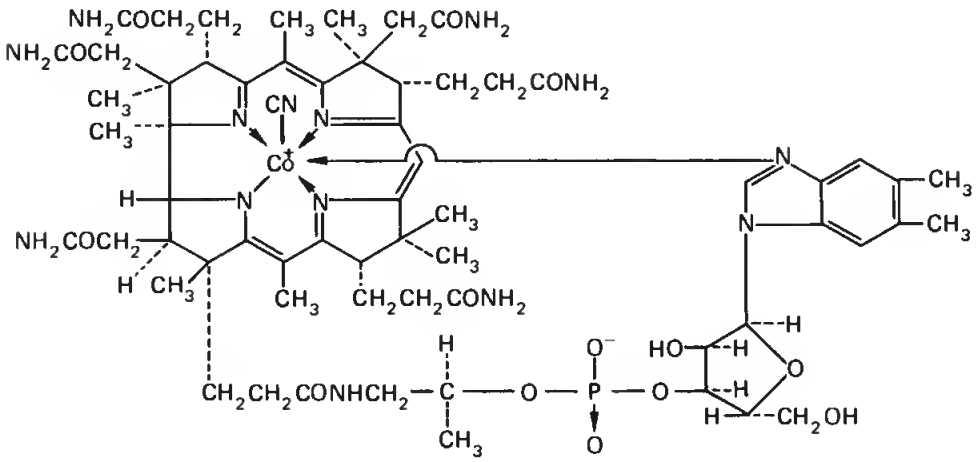
comparison with colecalciferol RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.48 ± 0.03.

CYANOCOBALAMINUM
CYANOCOBALAMIN

Molecular formula. $C_{63}H_{88}CoN_{14}O_{14}P$

Relative molecular mass. 1355

Graphic formula.



Chemical name. α-(5,6-Dimethylbenzimidazol-2-yl)cobamide cyanide; CAS Reg. No. 68-19-9.

Other name. Vitamin B_{12}.

Description. Dark red crystals or a red, crystalline powder; odourless.

Solubility. Soluble in 80 parts of water; soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R, and ether R.

Category. Haemopoietic.

Storage. Cyanocobalamin should be kept in a tightly closed container, protected from light.

Additional information. The anhydrous form of Cyanocobalamin is highly hygroscopic.

Requirements

Definition. Cyanocobalamin contains not less than 96.0% and not more than 102.0% of $C_{63}H_{88}CoN_{14}O_{14}P$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 20 µg/ml solution, when observed between 230 nm and 600 nm, exhibits 3 maxima at about 278 nm, 361 nm and 550 nm; the ratio of the absorbance of a 1-cm layer at 361 nm to that at 278 nm is between 1.70 and 1.90, and the ratio of the absorbance at 361 nm to that at 550 nm is between 3.15 and 3.45.

B. Mix 1 mg with about 10 mg of potassium sulfate R and 2 drops of sulfuric acid (~100 g/l) TS, heat the mixture carefully to redness until fused. Cool, break up the mass with a glass rod, and dissolve in 3 ml of water by boiling. Add 1 drop of phenolphthalein/ethanol TS and, drop by drop, sodium hydroxide (~80 g/l) TS until the solution is just pink. Add 0.5 g of sodium acetate R, 0.5 ml of acetic acid (~60 g/l) TS, and 0.5 ml of 1-nitroso-2-naphthol-3,6-disodium disulfonate (2 g/l) TS; a red or orange-red colour appears immediately. Add 0.5 ml of hydrochloric acid (~250 g/l) TS and boil for 1 minute; the red colour persists.

Clarity of solution. A solution of 20 mg in 10 ml of water is clear.

Loss on drying. Dry to constant weight at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 120 mg/g.

Pseudocyanocobalamin. Dissolve 1 mg in 20 ml of water. Transfer the solution to a small separator, add 5 ml of a mixture of equal volumes of carbon tetrachloride R and freshly distilled *o*-cresol R, and shake well for about 1 minute. Allow to separate, draw off the lower layer into a second small separator, add a mixture of 2.5 ml of sulfuric acid (~570 g/l) TS and 2.5 ml of water, shake well and allow to separate completely (the separation of the layers may be facilitated by centrifuging). Prepare a reference solution containing 1.5 ml of potassium permanganate (0.002 mol/l) VS in 250 ml of water. The separated upper layer of the test solution is colourless or not more intensely coloured than the reference solution when compared as described under 1.11 Colour of liquids.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using equal parts of silica gel R1 and kieselguhr R1 as the coating substance and a mixture of 15 volumes of chloroform R, 10 volumes

of methanol R, and 3 volumes of ammonia (~100 g/l) TS as the mobile phase. Carry out all operations protected from light. Apply separately to the plate 10 µl of each of 3 solutions containing (A) 5.0 mg of the test substance per ml, (B) 0.20 mg of the test substance per ml, and (C) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot is not more intense than that obtained with solution B. Not more than one spot obtained with solution A, other than the principal spot, is more intense than that obtained with solution C.

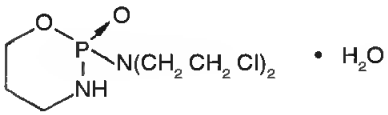
Assay. Dissolve about 0.03 g, accurately weighed, in sufficient water to produce 1000 ml. Determine the absorbance of this solution in a 1-cm layer at the maximum at about 361 nm and calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$, using the absorptivity value of 20.7 ($E^{1\%}_{1\,cm} = 207$).

CYCLOPHOSPHAMIDUM
CYCLOPHOSPHAMIDE

Molecular formula. $C_7H_{15}Cl_2N_2O_2P,H_2O$

Relative molecular mass. 279.1

Graphic formula.



Chemical name. 2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate; *N,N*-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide monohydrate; CAS Reg. No. 6055-19-2 (monohydrate).

Other name. Cyclophosphanum.

Description. A white, crystalline powder.

Solubility. Soluble in water; freely soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Cytotoxic drug.

Storage. Cyclophosphamide should be kept in a tightly closed container and stored at a temperature between 2 and 30 °C.

Additional information. CAUTION: Cyclophosphamide must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Cyclophosphamide contains not less than 98.0% and not more than 101.0% of $C_7H_{15}Cl_2N_2O_2P$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 0.1 g in 10 ml of water and add 5 ml of silver nitrate (40 g/l) TS; no precipitate is produced. Boil; a white precipitate is produced, which is insoluble in nitric acid (~130 g/l) TS but soluble in ammonia (~100 g/l) TS from which it is reprecipitated by the addition of nitric acid (~130 g/l) TS.

B. Dissolve 20 mg in 1 ml of sulfuric acid (~100 g/l) TS and heat until white fumes are evolved. After cooling, add 5 ml of water and shake. Neutralize with ammonia (~100 g/l) TS, then acidify with nitric acid (~130 g/l) TS; this test yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

Melting range. 49–53 °C, determined without previous drying.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.3 g of the substance; the water content is not less than 55 mg/g and not more than 70 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, determined 30 minutes after its preparation, 4.0–7.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 50 volumes of benzene R, 25 volumes of chloroform R, and 25 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions: (A) 25 mg of the test substance per ml of chloroform R and (B) 0.125 g of the test substance dissolved in 5.0 ml of water, boiled under a reflux condenser for 30 minutes, and then cooled to 20 °C. After removing the plate from the chromatographic chamber, allow it to dry in air and spray it with triketohydrindene/methanol TS. Examine the chromatogram in daylight. With

solution B, a pale violet spot is obtained at an R_f value between 0.10 and 0.25; other spots could also appear. With solution A, a brown-violet spot is obtained with an R_f value between 3.50 and 5.50, and no other spot is obtained above this spot.

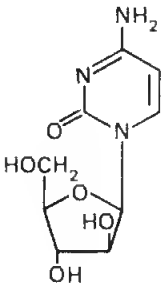
Assay. To about 0.2 g, accurately weighed, add 20 ml of potassium hydroxide/ethanol (0.5 mol/l) VS. Boil under a reflux condenser for 1 hour, cool, then add 30 ml of water, 3 ml of nitric acid (~1000 g/l) TS and 20.0 ml of silver nitrate (0.1 mol/l) VS. Shake the flask, add 5 ml of diethyl phthalate R and titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS, using 5 ml of ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$.

CYTARABINUM
CYTARABINE

Molecular formula. $C_9H_{13}N_3O_5$

Relative molecular mass. 243.2

Graphic formula.



Chemical name. 1-β-D-Arabinofuranosylcytosine; 4-amino-1-β-D-arabinofuranosyl-2(1*H*)-pyrimidone; CAS Reg. No. 147-94-4.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Category. Cytotoxic drug.

Storage. Cytarabine should be kept in a well-closed container, protected from light, and stored at a temperature not exceeding 15 °C.

Additional information. CAUTION: Cytarabine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Cytarabine contains not less than 99.0% and not more than 100.5% of $C_9H_{13}N_3O_5$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from cytarabine RS or with the *reference spectrum* of cytarabine.

B. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.55.

Specific optical rotation. Use a 10 mg/ml solution; $[\alpha]_D^{20\,°C}$ = +154 to +160 °.

Sulfated ash. Not more 5.0 mg/g.

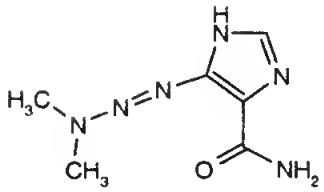
Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 13 volumes of ethylmethylketone R, 4 volumes of acetone R, and 3 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions containing (A) 40 mg of the test substance per ml, (B) 0.20 mg of undine R per ml, and (C) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A with an R_f value of about 1.1, compared with the spot obtained with solution B, is not more intense than that obtained with solution B. Any other spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator and titrate

with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.32 mg of $C_9H_{13}N_3O_5$.

DACARBAZINUM
DACARBAZINE



$C_6H_{10}N_6O$

Relative molecular mass. 182.2

Chemical name. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide; 5-(3,3-dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide; CAS Reg. No. 4342-03-4.

Description. A colourless or pale yellow, crystalline powder.

Solubility. Slightly soluble in water and ethanol (~750 g/l) TS.

Category. Cytotoxic drug.

Storage. Dacarbazine should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 8 °C.

Additional information. *CAUTION*: Dacarbazine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Dacarbazine contains not less than **97.0%** and not more than **102.0%** of $C_6H_{10}N_6O$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the

spectrum obtained from dacarbazine RS or with the *reference spectrum* of dacarbazine.

B. The absorption spectrum of a 6 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 323 nm and a pronounced shoulder at 275 nm. The absorbance of a 1-cm layer at the maximum wavelength of 323 nm is about 0.64.

C. Dissolve 25 mg in 5 ml of water, add 1 drop of cobalt(II) chloride (30 g/l) TS and 1 drop of ammonia (~100 g/l) TS; a violet-red solution is produced.

D. Dissolve 25 mg in 5 ml of hydrochloric acid (~70 g/l) TS, add about 0.2 g of zinc R powder and allow to stand for 5 minutes. Filter, and to the filtrate add 3 drops of sodium nitrite (10 g/l) TS and 0.5 ml of ammonium sulfamate (5 g/l) TS. After the reaction has subsided add 5 drops of *N*-(1-naphthyl)ethylenediamine hydrochloride/ethanol TS; a deep red solution is produced.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of citric acid (20 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 60 °C to constant mass under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and 5 volumes of 1-butanol R, 2 volumes of water and 1 volume of acetic acid (~300 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of the 3 following solutions in methanol R containing (A) 0.04 g of Dacarbazine per ml, (B) 0.4 mg of dacarbazine related compound A RS per ml, and (C) 0.4 mg of dacarbazine related compound B RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

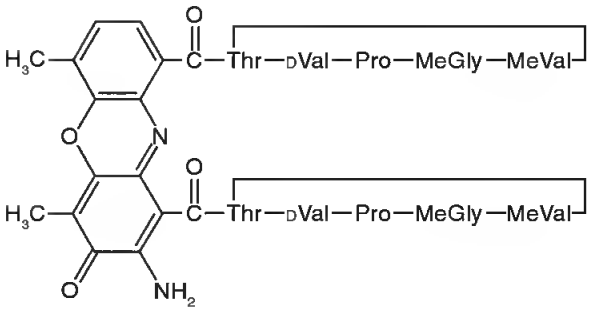
Any spot obtained with solution A, other than the principal spot, is not more intense or greater in size than that obtained with solution B (1%) and solution C (1%).

Assay

- The solutions must be protected from light throughout the assay.

Dissolve about 30 mg, accurately weighed, in sufficient hydrochloric acid (0.1 mol/l) VS to produce 50 ml of stock solution. For solution S_1 dilute 1.0 ml of the stock solution to 100 ml with hydrochloric acid (0.1 mol/l) VS. For solution S_2 dilute a further 1.0 ml aliquot of the stock solution to 100 ml with phosphate buffer, pH 7.0, TS. Measure the absorbance of a 1-cm layer of solution S_1 at the maximum at about 323 nm against a solvent cell containing hydrochloric acid (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer of solution S_2 at the maximum at about 329 nm against a solvent cell containing phosphate buffer, pH 7.0, TS. Calculate the percentage content of $C_6H_{10}N_6O$.

DACTINOMYCINUM
DACTINOMYCIN



$C_{62}H_{86}N_{12}O_{16}$

Relative molecular mass. 1255

Chemical name. Actinomycin D; CAS Reg. No. 50-76-0.

Description. An orange-red to red, crystalline powder.

Solubility. Soluble in water at 10 °C and slightly soluble in water at 37 °C; freely soluble in ethanol (~750 g/l) TS and methanol R; very slightly soluble in ether R.

Category. Cytotoxic drug.

Storage. Dactinomycin should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 40 °C.

Additional information. Dactinomycin is hygroscopic and is affected by light and heat.

CAUTION. Dactinomycin must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Dactinomycin contains not less than **95.0%** and not more than the equivalent of **103.0%** of $C_{62}H_{86}N_{12}O_{16}$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 25 µg/ml solution in methanol R, when observed between 220 nm and 500 nm, exhibits 2 maxima at about 240 nm and 445 nm. The absorbance of a 1-cm layer at the maximum wavelength of 445 nm is about 0.83; the ratio of the absorbance at 240 nm to that at 445 nm is between 1.30 and 1.50.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 4 volumes of 1-butanol R, 2 volumes of water, and 1 volume of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in acetone R containing (A) 10 mg of Dactinomycin per ml and (B) 10 mg of dactinomycin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Add 1 mg to a solution of 10 mg of paraformaldehyde R in 1 ml of sulfuric acid (~1760 g/l) TS; a red-violet colour is produced.

Melting range. 235–237 °C.

Specific optical rotation. Use a 1.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -292$ to -317 °.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

pH value. pH of a saturated solution, 5.5–7.0.

Assay. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column, length 30 cm, internal diameter 3.9 mm, packed with porous silica gel or ceramic microparticles having a diameter of

5–10 µm, the surface of which has been modified with chemically bonded octadecylsilyl groups.

As the mobile phase, use a mixture of 46 volumes of acetonitrile R, 25 volumes of sodium acetate (0.04 mol/l) VS and 25 volumes of acetic acid (0.07 mol/l) VS, filter through a membrane filter (porosity of 1 µm or finer) and degas the resulting solvent mixture. (*Note:* The concentration of acetonitrile may have to be adjusted to provide a suitable chromatogram and elution time.)

Prepare the following solutions immediately before use in the above-mentioned mobile phase, and store them protected from light. Weigh accurately for solution (A) about 1.2 mg of Dactinomycin per ml, and for solution (B) about 1.2 mg of dactinomycin RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer at a wavelength of about 254 nm. Make three replicate injections of solution B, each of 20 µl, to determine the peak responses. The relative standard deviation of the peaks is not more than 1.0%. Inject 20 µl of each of solutions A and B.

Measure the areas of the peak responses. (The retention time for dactinomycin is about 25 minutes.) Calculate the content in % of $C_{62}H_{86}N_{12}O_{16}$ using the following formula: (M_2/M_1) (A_1/A_2) 100, in which M_1 and M_2 are the concentrations , in mg per ml, of Dactinomycin being examined and the reference solution, and A_1 and A_2 are the areas of the peak responses of Dactinomycin and the reference substance, respectively.

Additional requirements for Dactinomycin for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 100.0 IU of endotoxin RS per mg.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using a solution in sterile water R containing 20 mg of Dactinomycin per ml.

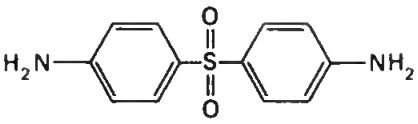
DAPSONUM

DAPSONE

Molecular formula. $C_{12}H_{12}N_2O_2S$

Relative molecular mass. 248.3

Graphic formula.



Chemical name. 4,4′-Sulfonyldianiline; 4,4′-sulfonylbis[benzenamine]; 4,4′-diaminodiphenylsulfone; CAS Reg. No. 80-08-0.

Description. A white or creamy white, crystalline powder; odourless.

Solubility. Soluble in 7000 parts of water and in 30 parts of ethanol (~750 g/l) TS; soluble in acetone R.

Category. Antileprotic.

Storage. Dapsone should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Dapsone is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Dapsone contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_2S$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 5.0 µg/ml solution in methanol R, when observed between 230 nm and 350 nm, exhibits maxima at about 260 nm and 295 nm; the absorbances of a 1-cm layer at the maximum wavelength of 260 nm and 295 nm are about 0.72 and 1.20, respectively.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. About 0.1 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a vivid red precipitate.

D. Melting temperature, about 178 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, but using an unlined chamber, silica gel R3 as the coating substance, and a mixture of 8 volumes of toluene R and 4 volumes of acetone R saturated with water as the mobile phase. Apply separately to the plate 10 µl of each of 5 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 10 mg of dapsone RS per ml, (C) 0.15 mg of the test substance per ml, (D) 20 µg of the test substance per ml and (E) 0.10 mg of 4,4′-thiodianiline RS per ml. The solution of 4,4′-thiodianiline RS should be freshly prepared. Pour the mobile phase into the chamber and insert the plate immediately, to avoid prior saturation of the chamber. After removing the plate from the chromatographic chamber, spray it with 4-dimethylaminocinnamaldehyde TS2. Heat the plate at 100 °C and examine the chromatogram in daylight. The spot obtained with solution C is more intense than any spot obtained with solution A, other than the principal spot, and in addition, not more than 2 among those secondary spots are more intense than the spot obtained with solution D. Moreover, there is no visible spot corresponding in position and appearance with that obtained with solution E.

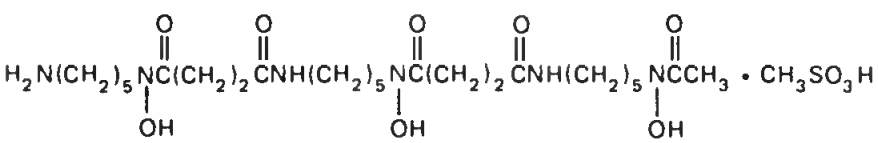
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.25 g, accurately weighed, dissolved in a mixture of 15 ml of water and 15 ml of hydrochloric acid (~70 g/l) TS and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

DEFEROXAMINI MESILAS
DEFEROXAMINE MESILATE

Molecular formula. $C_{25}H_{48}N_6O_8,CH_4O_3S$

Relative molecular mass. 656.8

Graphic formula.



Chemical name. *N*-[5-[3-[(5-Aminopentyl)hydroxycarbamoyl]propionamido] pentyl]-3-[[5-(*N*-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid monomethanesulfonate (salt); *N'*-[5-[[4-[[5-(acetylhydroxyamino)pentyl] amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide monomethanesulfonate (salt); CAS Reg. No. 138-14-7.

Other name. Desferrioxamine mesylate.

Description. A white to yellowish white powder; odourless or almost odourless.

Solubility. Soluble in 5 parts of water; soluble in ethanol (~750 g/l) TS; slightly soluble in methanol R; practically insoluble in ether R.

Category. Antidote to iron poisoning.

Storage. Deferoxamine mesilate should be kept in a well-closed container, protected from light, and stored at a temperature not exceeding 4 °C.

Requirements

Definition. Deferoxamine mesilate contains not less than 98.0% and not more than 102.0% of $C_{25}H_{48}N_6O_8,CH_4O_3S$, calculated with reference to the anhydrous substance.

Manufacture. The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Identity tests

A. Dissolve 5 mg in 5 ml of water, add 2 ml of trisodium orthophosphate (2 g/l) TS, mix, then add 1 ml of sodium 1,2-naphthoquinone-4-sulfonate (5 g/l) TS; a blackish brown colour is produced.

B. The titrated solution obtained in the assay is reddish brown in colour. To 5 ml of the titrated solution add 2 ml of benzyl alcohol R and shake; the colour is extracted. To a further 5 ml of the titrated solution add 2 ml of ether R and shake; the colour is not extracted.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 0.7 g in a mixture of 2 ml of nitric acid (~130 g/l) TS, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.35 mg/g.

Sulfates. Dissolve 0.85 g in 40 ml of water, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.6 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear; measure the absorbance of the solution in a 1-cm layer at 420 nm; not more than 0.10.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 20 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 3.5–6.0.

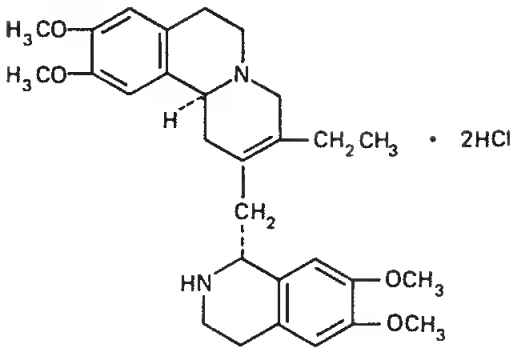
Assay. Dissolve about 0.3 g, accurately weighed, in 15 ml of water and add 2 ml of sulfuric acid (0.05 mol/l) VS. Titrate slowly with ferric ammonium sulfate (0.1 mol/l) VS, determining the endpoint potentiometrically using a platinum electrode and a calomel reference electrode. Each ml of ferric ammonium sulfate (0.1 mol/l) VS is equivalent to 65.68 mg of $C_{25}H_{48}N_6O_8,CH_4O_3S$. (Keep the titrated solution for identity test B.)

DEHYDROEMETINI DIHYDROCHLORIDUM
DEHYDROEMETINE DIHYDROCHLORIDE

Molecular formula. $C_{29}H_{38}N_2O_4,2HCl$

Relative molecular mass. 551.6

Graphic formula.



Chemical name. (±)-2,3-Didehydroemetine dihydrochloride; (±)-2,3-didehydro-6′,7′,10,11-tetramethoxyemetan dihydrochloride; (±)-(11b*R**)-3-ethyl-1,6,7,11b-tetrahydro-9,10-dimethoxy-1-[[(1b*S**)-1,2,3,4-tetrahydro-6,7-dimethoxy-1-isoquinolyl]methyl]-4*H*-benzo[*a*]quinolizine dihydrochloride; CAS Reg. No. 3317-75-7.

Description. A white to yellowish, crystalline powder; odourless.

Solubility. Sparingly soluble in water; soluble in methanol R.

Category. Antiamoebic drug.

Storage. Dehydroemetine dihydrochloride should be kept in a tightly closed container.

Requirements

Definition. Dehydroemetine dihydrochloride contains not less than 98.0% and not more than 101.0% of $C_{29}H_{38}N_2O_4,2HCl$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.040 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 240 nm and 350 nm, exhibits a maximum at about 282 nm. The absorbance of a 1-cm layer at this wavelength is about 0.49.

B. Sprinkle a small quantity of the powdered substance on the surface of 1 ml of sulfuric acid (~1760 g/l) TS containing 5 mg of molybdenum trioxide R; a bright green colour is produced.

C. A 0.1 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Clarity and colour of solution. A solution of 0.30 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 70 mg/g.

pH value. pH of a 30 mg/ml solution, 3.5–5.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of ethyl acetate R and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 20 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of emetine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with mercuric acetate/acetic acid TS, heat it at 120 °C for 10 minutes, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B or solution C, as appropriate.

Assay. Dissolve about 0.4 g, accurately weighed, in 75 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.58 mg of $C_{29}H_{38}N_2O_4,2HCl$.

DEXAMETHASONI ACETAS

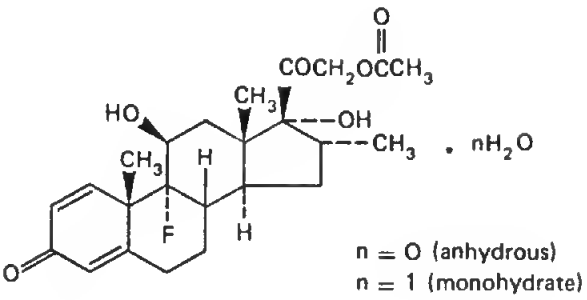
DEXAMETHASONE ACETATE

Dexamethasone acetate, anhydrous
Dexamethasone acetate monohydrate

Molecular formula. $C_{24}H_{31}FO_6$ (anhydrous); $C_{24}H_{31}FO_6,H_2O$ (monohydrate).

Relative molecular mass. 434.5 (anhydrous); 452.5 (monohydrate).

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate; 21-(acetyloxy)-9-fluoro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione; CAS Reg. No. 1177-87-3 (anhydrous).

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate monohydrate; 21-(acetyloxy)-9-fluoro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione monohydrate; CAS Reg. No. 55812-90-3 (monohydrate).

Description. A white or almost white powder, odourless.

Solubility. Practically insoluble in water; soluble in 40 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Adrenoglucocorticoid.

Storage. Dexamethasone acetate should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container of Dexamethasone acetate should state whether the substance is the monohydrate or is in the anhydrous form.

Requirements

Definition. Dexamethasone acetate contains not less than 96.0% and not more than 104.0% of $C_{24}H_{31}FO_6$, calculated with reference to the dried substance.

Identity tests

- Either tests A, B, C and E, or tests B, C, D and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For the anhydrous form the infrared absorption spectrum is

concordant with the spectrum obtained from dexamethasone acetate RS or with the *reference spectrum* of dexamethasone acetate. For the monohydrate the infrared absorption spectrum is concordant with the spectrum obtained from dexamethasone acetate monohydrate RS or with the *reference spectrum* of dexamethasone acetate monohydrate.

B. Dissolve 22 mg in 20 ml of ethanol (~750 g/l) TS and dilute 2 ml to 20 ml with the same solvent. To 2 ml of this solution placed in a stoppered test-tube add 10 ml of phenylhydrazine/sulfuric acid TS, mix, heat in a water-bath at 60 °C for 20 minutes and cool immediately. The absorbance of a 1-cm layer at the maximum at about 423 nm is not less than 0.42 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

C. See the test described below under "Related steroids". The principal spots obtained with solutions A and C correspond in position with that obtained with solution B. In addition the appearance and intensity of the principal spot obtained with solution A corresponds with that obtained with solution B.

D. Carry out the combustion as described under 2.4 Oxygen flask method, using 7 mg of the test substance and a mixture of 0.5 ml of sodium hydroxide (0.01 mol/l) VS and 20 ml of water as the absorbing liquid. When the process is complete, add 0.1 ml to a mixture of 0.1 ml of freshly prepared sodium alizarinsulfonate (1 g/l) TS and 0.1 ml of zirconyl nitrate TS; the red colour of the solution changes to clear yellow.

E. Heat 0.05 g with 2 ml of potassium hydroxide/ethanol (0.5 mol/l) VS in a water-bath for 5 minutes. Cool, add 2 ml of sulfuric acid (~700 g/l) TS, and boil gently for 1 minute; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +82$ to +88 °.

Sulfated ash. Weigh 0.1 g and use a platinum dish; not more than 5.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury). For the anhydrous form use about 0.5 g of the substance; it loses not more than 5.0 mg/g. For the monohydrate use about 0.15 g of the substance; it loses not less than 35 mg/g and not more than 45 mg/g.

Related steroids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of

methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 15 mg of dexamethasone acetate RS per ml; also apply to the plate 2 µl of a third solution (C) composed of a mixture of equal volumes of solutions A and B and 1 µl of a fourth solution (D) containing 0.15 mg of the test substance per ml in the same solvent mixture as used for solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated and heat at 105 °C for 10 minutes; allow to cool, spray with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D.

Assay

- The solutions must be protected from light throughout the assay.

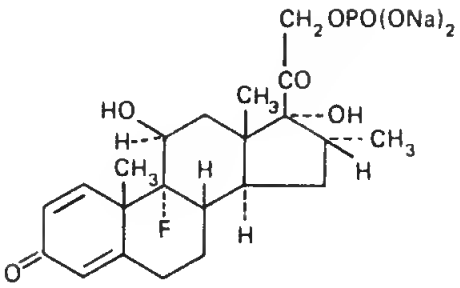
Dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Dilute 20 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS and displace the air with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. Calculate the amount of $C_{24}H_{31}FO_6$ in the substance being tested by comparison with dexamethasone acetate RS, similarly and concurrently examined.

DEXAMETHASONI NATRII PHOSPHAS
DEXAMETHASONE SODIUM PHOSPHATE

Molecular formula. $C_{22}H_{28}FNa_2O_8P$

Relative molecular mass. 516.4

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-(dihydrogen phosphate) disodium salt; 9-fluoro-11β,17-dihydroxy-16α-methyl-21-(phosphonooxy)pregna-1,4-diene-3,20-dione disodium salt; CAS Reg. No. 2392-39-4.

Description. A white or almost white, crystalline powder; odourless or with a slight odour of ethanol.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Adrenal hormone.

Storage. Dexamethasone sodium phosphate should be kept in a tightly closed container, protected from light.

Additional information. Dexamethasone sodium phosphate is very hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Dexamethasone sodium phosphate contains not less than 96.0% and not more than 103.0% of $C_{22}H_{28}FNa_2O_8P$, calculated with reference to the anhydrous and ethanol-free substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a freshly prepared mixture of 3 volumes of 1-butanol R, 1 volume of acetic anhydride R, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of 4 solutions in methanol R containing (A) 2.5 mg of the test substance per ml, (B) 2.5 mg of dexamethasone sodium phosphate RS per ml, (C) a mixture of equal volumes of solutions A and B, and (D) equal volumes of solution

A and a solution of 2.5 mg of prednisolone sodium phosphate RS per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with a mixture of 10 ml of sulfuric acid (~1760 g/l) TS and 90 ml of ethanol (~750 g/l) TS, heat it at 120 °C for 10 minutes, allow it to cool, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The principal spot obtained with solution C appears as a single compact spot, whereas the chromatogram of solution D shows 2 closely running spots.

B. Place 0.5 ml of chromic acid TS in a small test-tube and heat in a water-bath for 5 minutes; the solution wets the sides of the tube but there is no greasiness. Add about 3 mg of the test substance and again heat in a water-bath for 5 minutes; the solution no longer wets the sides of the tube.

C. Heat carefully 0.04 g with 2 ml of sulfuric acid (~1760 g/l) TS until white fumes are evolved, add drop by drop nitric acid (~1000 g/l) TS until oxidation is complete, and cool. Add 2 ml of water, heat until white fumes are again evolved, cool, add 10 ml of water, and neutralize with ammonia (~100 g/l) TS, using pH-indicator paper R. Keep half of the solution for test D. The remaining solution yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

D. The solution prepared in test C yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the anhydrous and ethanol-free substance; $[\alpha]_D^{20\,°C} = +74$ to $+82\,°$.

Clarity of solution. A solution of 0.10 g in 10 ml of carbon-dioxide-free water R is clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.3 g of the substance. The sum of the contents of water and ethanol (described below), both calculated in mg/g, is not more than 160 mg/g.

Ethanol. Carry out the test as described under 1.14.5 Gas chromatography, using 3 solutions in water containing (1) a mixture of 10 µl of 1-propanol R per ml serving as an internal standard and 10 µl of dehydrated ethanol R per ml, (2) 0.10 g of the test substance per ml, and (3) a mixture of 0.10 g of the test substance and 10 µl of the internal standard per ml. It may be necessary to adjust the content of dehydrated ethanol R in solution (1) to produce a peak of similar height to the corresponding peak in the chromatogram obtained with solution (2).

For the procedure use a column 1.5 m long and 4 mm in internal diameter packed with porous polymer beads (particle size 80–100 µm from a commercial source, is suitable). Maintain the column at 135 °C, use nitrogen R as the carrier gas and a flame ionization detector.

Calculate the content of ethanol in mg/g, assuming the weight per ml at 20 °C to be 0.790 g; not more than 80 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 7.5–10.5.

Free dexamethasone and other related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and methanol R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per ml, and (B) 0.20 mg of dexamethasone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 5 minutes, spray it with a solution of 3 g of zinc chloride R in 10 ml of methanol R, heat it at about 125 °C for 1 hour, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.2 g, accurately weighed, in sufficient water to produce 200 ml. Dilute 5 ml to 250 ml with water and measure the absorbance of this solution in a 1-cm layer at the maximum at about 241 nm. Calculate the content of $C_{22}H_{28}FNa_2O_8P$, using the absorptivity value of 29.7 ($A^{1\%}_{1\,cm}$ = 397).

Additional requirements for Dexamethasone sodium phosphate for parenteral use

Complies with the monograph for "Parenteral preparations".

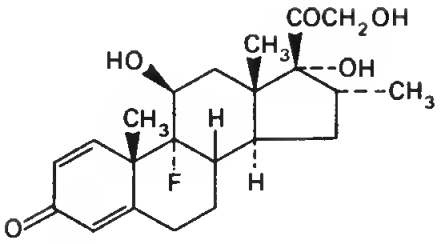
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 31.3 IU of endotoxin RS per mg.

DEXAMETHASONUM
DEXAMETHASONE

Molecular formula. $C_{22}H_{29}FO_5$

Relative molecular mass. 392.5

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione; CAS Reg. No. 50-02-2.

Description. Colourless crystals or a white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Adrenoglucocorticoid.

Storage. Dexamethasone should be kept in a tightly closed container, protected from light.

Requirements

Definition. Dexamethasone contains not less than 96.0% and not more than 104.0% of $C_{22}H_{29}FO_5$, calculated with reference to the dried substance.

Identity tests

- Either tests A, B and C, or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dexamethasone RS or with the *reference spectrum* of dexamethasone.

B. Dissolve 20 mg in 20 ml of ethanol (~750 g/l) TS and dilute 2 ml to 20 ml with the same solvent. To 2 ml of this solution placed in a stoppered test-tube add 10 ml of phenylhydrazine/sulfuric acid TS, mix, heat in a water-bath at 60 °C for 20 minutes, and cool immediately. The absorbance of a 1-cm layer at the maximum at about 423 nm is not less than 0.42 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

C. See the test described below under "Related steroids". The principal spots obtained with solutions A and C correspond in position with that obtained

with solution B. In addition the appearance and intensity of the principal spot obtained with solution A corresponds with that obtained with solution B.

D. Carry out the combustion as described under 2.4 Oxygen flask method, using 7 mg of the test substance and a mixture of 0.5 ml of sodium hydroxide (0.01 mol/l) VS and 20 ml of water as the absorbing liquid. When the process is complete add 0.1 ml to a mixture of 0.1 ml of freshly prepared sodium alizarinsulfonate (1 g/l) TS and 0.1 ml of zirconyl nitrate TS; the red colour of the solution changes to clear yellow.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +72$ to +80 °.

Sulfated ash. Weigh 0.1 g and use a platinum dish; not more than 5.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related steroids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 15 mg of dexamethasone RS per ml; also apply to the plate 2 µl of a third solution (C) composed of a mixture of equal volumes of solutions A and B and 1 µl of a fourth solution (D) containing 0.15 mg of the test substance per ml in the same solvent mixture as used for solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated and heat at 105 °C for 10 minutes; allow to cool, spray with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D.

Assay

- The solutions must be protected from light throughout the assay.

Dissolve about 20 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Dilute 20 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS, and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again

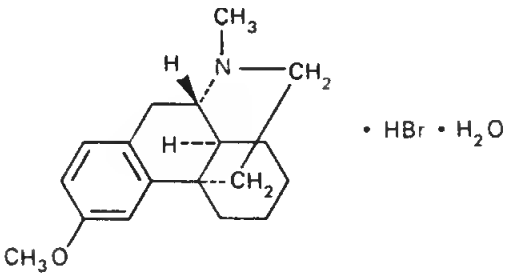
displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. Calculate the amount of $C_{22}H_{29}FO_5$ in the substance being tested by comparison with dexamethasone RS, similarly and concurrently examined.

DEXTROMETHORPHANI HYDROBROMIDUM
DEXTROMETHORPHAN HYDROBROMIDE

Molecular formula. $C_{18}H_{25}NO,HBr,H_2O$

Relative molecular mass. 370.3

Graphic formula.



Chemical name. (+)-3-Methoxy-17-methyl-9α,13α-14α-morphinan hydrobromide monohydrate; (+)-*cis*-1,3,4,9,10,10a-hexahydro-6-methoxy-11-methyl-2*H*-10,4a-iminoethanophenanthrene hydrobromide monohydrate; CAS Reg. No. 6700-34-1 (monohydrate).

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antitussive drug.

Storage. Dextromethorphan hydrobromide should be kept in a well-closed container.

Requirements

Definition. Dextromethorphan hydrobromide contains not less than 98.0% and not more than 101.0% of $C_{18}H_{25}NO,HBr$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and E or tests B, C, D and E may be applied.

A. Dry a small quantity of the test substance for 4 hours under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R, and carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dextromethorphan hydrobromide RS similarly prepared or with the *reference spectrum* of dextromethorphan hydrobromide.

B. The absorption spectrum of a 0.10 mg/ml solution in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.59.

C. Dissolve 0.05 g in 2 ml of sulfuric acid (~100 g/l) TS. Add 1 ml of mercury/nitric acid TS drop by drop while shaking; a white, crystalline precipitate in the form of platelets is produced, and the solution does not immediately turn red. Heat on a water-bath for about 10 minutes; a yellow to red colour develops.

D. Melting temperature, about 125 °C with decomposition.

E. To a 5 mg/ml solution add 0.25 ml of nitric acid (~130 g/l) TS; this test yields reaction B described under 2.1 General identification tests as characteristic of bromides.

Specific optical rotation. Use a 20 mg/ml solution in hydrochloric acid (0.1 mol/l) VS and calculated with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +28.0$ to $+30°$.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 35 mg/g and not more than 55 mg/g.

pH value. Dissolve 0.4 g in carbon-dioxide-free water R using gentle heat, dilute to 20 ml with the same solvent and measure the pH at 20 °C; the value lies between 5.2 and 6.5.

Dimethylaniline. Dissolve 0.5 g in 15 ml of water using gentle heat, cool, and add 4 ml of acetic acid (~60 g/l) TS, 1 ml of sodium nitrite (10 g/l) TS, and sufficient water to produce 25 ml. Prepare similarly a reference solution containing 5 µg of *N,N*-dimethylaniline R in 25 ml. The colour produced in the test solution is not more intense than that produced in the reference solution when compared as described under 1.11 Colour of liquids; the dimethylaniline content is not more than 10 µg/g.

Phenolic substances. To 5 mg add 1 drop of hydrochloric acid (~70 g/l) TS, 1 ml of water, and 0.2 ml of ferric chloride (50 g/l) TS. Mix, add 0.2 ml of potassium ferricyanide (50 g/l) TS, dilute to 5 ml with water, shake well, and allow to stand for 15 minutes; the solution is yellowish brown and shows no greenish or blue colour.

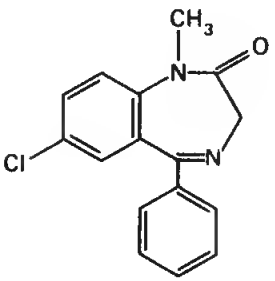
Assay. Dissolve about 0.5 g, accurately weighed, in 40 ml of glacial acetic acid R1, and add 10 ml of mercuric acetate/acetic acid TS, warming slightly if necessary to effect solution. Titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 35.23 mg of $C_{18}H_{25}NO$, HBr.

DIAZEPAMUM
DIAZEPAM

Molecular formula. $C_{16}H_{13}ClN_2O$

Relative molecular mass. 284.7

Graphic formula.



Chemical name. 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one; CAS Reg. No. 439-14-5.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; soluble in ethanol (~750 g/l) TS.

Category. Tranquillizer.

Storage. Diazepam should be kept in a well-closed container, protected from light.

Requirements

Definition. Diazepam contains not less than 99.0% and not more than 101.0% of $C_{16}H_{13}ClN_2O$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.
- For tests B and C use low-actinic glassware and measure within 30 minutes.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of diazepam.

B. The absorption spectrum of an 8.0 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits maxima at about 241 nm and 286 nm; the absorbances of a 1-cm layer at the maximum wavelengths of 241 nm and 286 nm are about 0.80 and 0.38, respectively (preferably use 2-cm cells for the measurements and calculate the absorbances for 1-cm layers).

C. The absorption spectrum of a 0.030 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 325 nm and 400 nm, exhibits a maximum at about 362 nm; the absorbance of a 1-cm layer at this wavelength is about 0.44 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

D. Carry out the combustion as described under 2.4 Oxygen flask method, using 20 mg of the test substance and 5 ml of sodium hydroxide (~80 g/l) TS as the absorbing liquid. When the process is complete, acidify with sulfuric acid (~100 g/l) TS and boil gently for 2 minutes; the solution yields reaction

A, described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 131–135 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 50 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test in subdued light as described under 1.14.1 Thin-layer chromatography, using silical gel R2 as the coating substance and a mixture of 1 volume of dehydrated ethanol R and 24 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of 2 freshly prepared solutions in chloroform R containing (A) 0.20 g of the test substance per ml and (B) 0.10 mg of 5-chloro-2-methylaminobenzophenone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 28.47 mg of $C_{16}H_{13}ClN_2O$.

Additional requirements for Diazepam for parenteral use

Complies with the monograph for "Parenteral preparations".

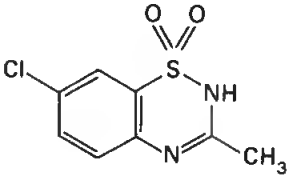
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 11.6 IU of endotoxin RS per mg.

DIAZOXIDUM
DIAZOXIDE

Molecular formula. $C_8H_7ClN_2O_2S$

Relative molecular mass. 230.7

Graphic formula.



Chemical name. 7-Chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide; CAS Reg. No. 364-98-7.

Description. A white, or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water and ether R; freely soluble in dimethylformamide R; slightly soluble in ethanol (~750 g/l) TS.

Category. Antihypertensive.

Storage. Diazoxide should be kept in a well-closed container.

Requirements

Definition. Diazoxide contains not less than 98.0% and not more than 101.0% of $C_8H_7ClN_2O_2S$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diazoxide RS or with the *reference spectrum* of diazoxide.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 17 volumes of ethyl acetate R, 4 volumes of methanol R, and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in sodium hydroxide (0.1 mol/l) VS containing (A) 15 mg of the test substance per ml, (B) 0.15 mg of the test substance per ml and (C) 0.15 mg of diazoxide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the odour of ammonia is no longer detectable, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

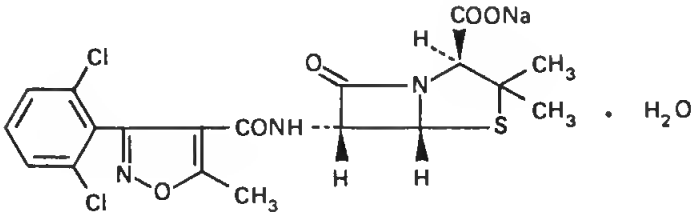
Assay. Dissolve 0.45 g, accurately weighed, in 100 ml of a mixture of 2 volumes of dimethylformamide R and 1 volume of water, and titrate with sodium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 23.07 mg of $C_8H_7ClN_2O_2S$.

DICLOXACILLINUM NATRICUM
DICLOXACILLIN SODIUM

Molecular formula. $C_{19}H_{16}Cl_2N_3NaO_5S,H_2O$

Relative molecular mass. 510.3

Graphic formula.



Chemical name. Monosodium (2*S*,5*R*,6*R*)-6-[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate monohydrate; monosodium [2*S*-(2α,5α,6β)]-6-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate;

monosodium [3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]penicillin monohydrate; CAS Reg. No. 13412-64-1 (monohydrate).

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/l) TS.

Category. Antibacterial drug.

Storage. Dicloxacillin sodium should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Dicloxacillin sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Dicloxacillin sodium contains not less than 88.0% of total penicillins calculated as dicloxacillin free acid ($C_{19}H_{17}Cl_2N_3O_5S$) and with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dicloxacillin sodium RS or with the *reference spectrum* of dicloxacillin sodium.

B. To 10 mg of paraformaldehyde R dissolved in 1 ml of sulfuric acid (~1760 g/l) TS add about 1 mg of the test substance; a colourless solution is produced. Heat the solution in a water-bath for 2 minutes and cool; the solution remains colourless (distinction from cloxacillin sodium).

C. Ignite 20 mg and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a 10 mg/ml solution, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +128 to +143 °.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not less than 30 mg/g and not more than 50 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5–7.5.

Chlorine. Carry out the combustion as described under 2.4 Oxygen flask method, but using 25 mg of the test substance and 10 ml of sodium hydroxide (0.1 mol/l) VS as the absorbing liquid. When the process is complete, transfer the resulting solution to a titration vessel, heat on a water-bath for 30 minutes, cool to room temperature, add 20 ml of nitric acid (~130 g/l) TS, and titrate with silver nitrate (0.01 mol/l) VS, determining the endpoint potentiometrically using a silver/silver chloride electrode system. Repeat the operation without the substance being tested. Each ml of silver nitrate (0.01 mol/l) VS is equivalent to 0.3546 mg of Cl. Calculate the total content of chlorine in mg/g and subtract from it the content of free chlorides as determined below; the content of chlorine is between 130 mg/g and 142 mg/g.

Free chlorides. Dissolve about 0.12 g, accurately weighed, in 10 ml of sodium hydroxide (0.1 mol/l) VS, add 20 ml of water, and heat on a water-bath for 30 minutes. Cool to room temperature, add 20 ml of nitric acid (~130 g/l) TS, and titrate with silver nitrate (0.01 mol/l) VS, determining the endpoint potentiometrically using a silver/silver chloride electrode system. Repeat the operation without the substance being tested. Each ml of silver nitrate (0.01 mol/l) VS is equivalent to 0.3546 mg of Cl; the content of free chlorides is not more than 5 mg/g.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A). To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 343 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{19}H_{16}Cl_2N_3NaO_5S$ in the substance being tested by comparison with dicloxacillin sodium RS, similarly and concurrently examined.

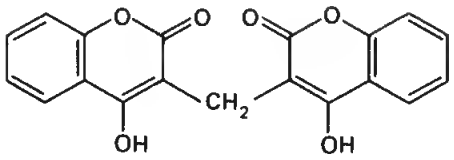
DICOUMAROLUM

DICOUMAROL

Molecular formula. $C_{19}H_{12}O_6$

Relative molecular mass. 336.3

Graphic formula.



Chemical name. 3,3′-Methylenebis[4-hydroxycoumarin]; 3,3′-methylenebis [4-hydroxy-2*H*-1-benzopyran-2-one]; CAS Reg. No. 66-76-2.

Description. A white or creamy white, crystalline powder; odour, characteristic, faint.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, and ether R.

Category. Anticoagulant.

Storage. Dicoumarol should be kept in a well-closed container, protected from light.

Requirements

Definition. Dicoumarol contains not less than 98.5% and not more than 101.0% of $C_{19}H_{12}O_6$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dicoumarol RS or with the *reference spectrum* of dicoumarol.

B. Fuse 0.2 g with 0.2 g of potassium hydroxide R, cool, stir with 5 ml of water, filter and acidify the filtrate with hydrochloric acid (~250 g/l) TS; a white, crystalline precipitate is obtained (salicylic acid). Retain the filtrate for test C.

C. To 1 ml of the filtrate from test B add 5 ml of water and a mixture of 1 drop of ferric chloride (25 g/l) TS and 2 drops of hydrochloric acid (~70 g/l) TS; a violet colour is produced.

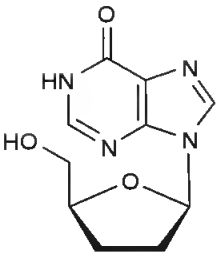
Sulfated ash. Not more than 2.5 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Shake 0.5 g with 10 ml of carbon-dioxide-free water R for 1 minute and filter; titrate the filtrate with sodium hydroxide (0.1 mol/l) VS, methyl red/ethanol TS being used as indicator; not more than 0.1 ml is required to obtain the midpoint of the indicator (orange).

Assay. Dissolve about 0.35 g, accurately weighed, in 40 ml of 1-butylamine R, add 5 drops of azo violet TS and titrate with lithium methoxide (0.1 mol/l) VS to a deep-blue endpoint, as described under 2.6 Non-aqueous titration, Method B. Each ml of lithium methoxide (0.1 mol/l) VS is equivalent to 16.82 mg of $C_{19}H_{12}O_6$.

DIDANOSINUM
DIDANOSINE



$C_{10}H_{12}N_4O_3$

Relative molecular mass. 236.2

Chemical name. 9-[(2*R*,5*S*)-5-(hydroxymethyl)tetrahydrofuran-2-yl]-1,9-dihydro-6*H*-purin-6-one; 9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one; 2′,3′-dideoxyinosine (DDI); CAS Reg. No. 69655-05-6.

Description. A white to almost white powder.

Solubility. Sparingly soluble in water; slightly soluble in methanol R and ethanol (95 per cent) R.

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Didanosine should be kept in a tightly closed container.

Requirements

Didanosine contains not less than **98.5%** and not more than **101.0%** of $C_{10}H_{12}N_4O_3$, calculated with reference to the dried substance.

Identity test

- Either tests A and B, or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of didanosine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of didanosine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120 °C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 210 nm and 300 nm, exhibits one maximum at about 250 nm; the specific absorbance ($A^{1\%}_{1\,cm}$) is between 435 to 485.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS or with the *reference spectrum* of didanosine.

If the spectra are not concordant, use didanosine RS. Dissolve the sample in a small amount of methanol R, evaporate to dryness and carry out the IR spectrum with the residue as mentioned above. Treat didanosine RS in the same way. The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -24$ to $-28\,°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 4 hours at 105 °C; it loses not more than 5.0 mg/g.

Related substances
Prepare fresh solutions and perform the tests without delay

Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm), packed with octadecylsilyl base-deactivated silica gel for chromatography R (5 µm).

Maintain the column temperature at 20–25 °C.

The mobile phases for gradient elution consist of a mixture of aqueous phase (Mobile phase A) and methanol (Mobile phase B), using the following conditions:

Mobile phase A: A 0.05 M solution of ammonium acetate R adjusted to pH 8.0 using ammonia (~260 g/l) TS.

Mobile phase B: Methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	92	8
18	92	8
25	70	30
45	70	30
50	92	8
60	92	8

Prepare the following solutions in a mixture of 92 volumes of mobile phase A and 8 volumes of mobile phase B (dissolution solvent).

For solution (1) dissolve 5.0 mg of hypoxanthine R in the dissolution solvent and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 20.0 ml with the same solvent. For solution (2) dissolve 5 mg of didanosine for system suitability RS (containing impurities A to F) in the dissolution solvent and dilute to 10 ml with the same solvent. For solution (3) dissolve 25 mg of the test substance in the dissolution solvent and dilute to 50.0 ml with the same solvent. For solution (4) dilute 5.0 ml of solution (3) to 50.0 ml with the dissolution solvent. Then dilute 5.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Use the chromatogram supplied with didanosine for system suitability RS and the chromatogram obtained with solution (2) to identify the peaks due to impurities A to F.

Inject 20 µl of solution (2). The test is not valid unless the resolution factor between the peaks due to impurity (C) (2′-deoxyinosine) and impurity D (3′-deoxyinosine) is greater than 2.5, if necessary reduce the amount of methanol in the mobile phase and adjust the proportion of aqueous phase pH 8.0 accordingly.

Inject separately 20 µl of solution (4) in replicate injections in the chromatographic system. The relative standard deviation for peak areas of didanosine in replicate injections of solution (4) is not more than 5.0%.

Inject separately 20 µl each of solutions (1) and (3) and 20 ml of dissolution solvent in the chromatographic system. Examine the mobile phase chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (2), the following peaks are eluted at the following relative retention with reference to didanosine (retention time about 13–15 min): impurity A about 0.3; impurity B about 0.4; impurity C about 0.44; impurity D about 0.48; impurity E about 0.5; impurity F about 0.8; impurity I about 1.4; impurity G about 1.6; impurity H about 2.0.

In the chromatogram obtained with solution (3) the area of any peak corresponding to impurity A (hypoxanthine) is not greater than the area of the principal peak obtained with solution (1) (0.5%). The area of any individual peak corresponding to impurities B, C, D, E, F or G is not greater than 0.2 times the area of the principal peak obtained with solution (4) (0.2%). The area of any other impurity peak is not greater than 0.1 times the area of the principal peak obtained with solution (4) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than the area of the principal peak obtained

with solution (4) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (4) (0.05%).

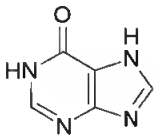
Assay

Dissolve about 0.200 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration; Method A determining the end point potentiometrically.

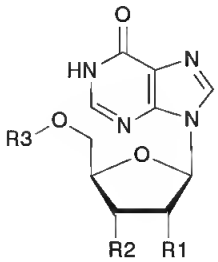
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 23.62 mg of $C_{10}H_{12}N_4O_3$.

Impurities

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.



A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine)

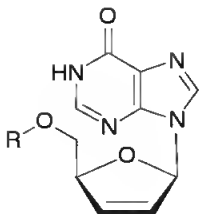


B. R1 = R2 = OH, R3 = H
9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine)

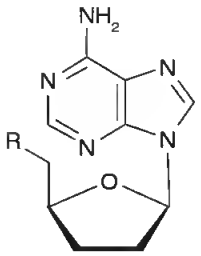
C. R1 = R3 = H, R2 = OH
9-(2-deoxy- β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′-deoxyinosine)

D. R1 = OH, R2 = R3 = H
9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (3′-deoxyinosine)

E. R1 + R2 = O, R3 = H
9-(2,3-anhydro-β-D-ribofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-anhydroinosine)

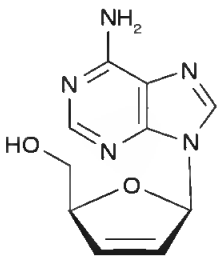


F. R = H
9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl]-1,9-dihydro-6*H*-purin-6-one;
(2′,3′-didehydro-2′,3′-dideoxyinosine)



G. R = OH
9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine
(2′,3′-dideoxyadenosine)

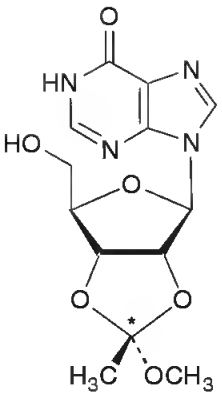
H. R = H
9-(2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine
(2′,3′,5′-trideoxyadenosine)



I. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-9*H*-purin-6-amine
(2′,3′-dideoxy-2′,3′-didehydroadenosine)

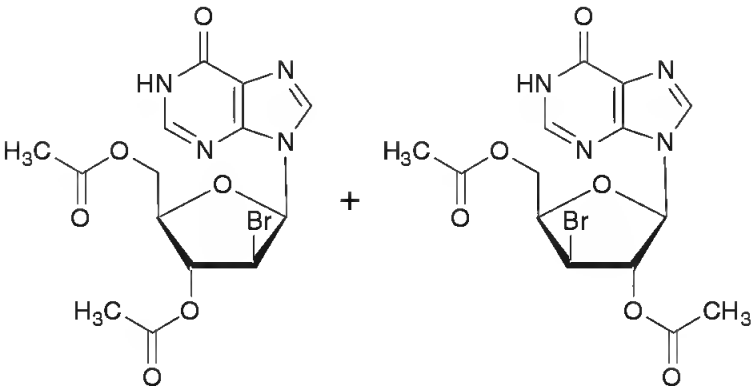
J. structure as shown for impurities B to E where R1 = R2 = H, R3 = CO-CH_3
9-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (didanosine acetate)

K. structure as shown for impurity F where R = CO-CH_3
9-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2′,3′-didehydrodidanosine acetate)



and epimer at C*

L. 9-[2,3-*O*-[(1*RS*)-1-methoxyethylene]-β-D-ribofuranosyl]-1,9-dihydro-6*H*-purin-6-one (2′,3′-*O*-(1-methoxyethylidene)inosine; ("dioxalane")



M. mixture of 9-(3,5-di-*O*-acetyl-2-bromo-2-deoxy-β-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one and 9-(2,5-di-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-1,9-dihydro-6*H*-purin-6-one ("bromoesters")

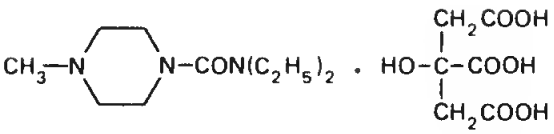
DIETHYLCARBAMAZINI DIHYDROGENOCITRAS

DIETHYLCARBAMAZINE DIHYDROGEN CITRATE

Molecular formula. $C_{10}H_{21}N_3O,C_6H_8O_7$ or $C_{16}H_{29}N_3O_8$

Relative molecular mass. 391.4

Graphic formula.



Chemical name. *N,N*-Diethyl-4-methyl-1-piperazinecarboxamide citrate (1 : 1); *N,N*-diethyl-4-methyl-1-piperazinecarboxamide 2-hydroxy-1,2,3-propanetricarboxylate (1 : 1); CAS Reg. No. 1642-54-2.

Description. A white, crystalline powder; odourless or almost odourless.

Solubility. Very soluble in water; soluble in 35 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Filaricide.

Storage. Diethylcarbamazine dihydrogen citrate should be kept in a tightly closed container, protected from light.

Additional information. Diethylcarbamazine dihydrogen citrate is hygroscopic; it has an acid and bitter taste. Even in the absence of light, Diethylcarbamazine dihydrogen citrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Diethylcarbamazine dihydrogen citrate contains not less than 98.0% and not more than 101.0% of $C_{10}H_{21}N_3O,C_6H_8O_7$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and D or tests B and C may be applied.

A. Dissolve 0.05 g in 25 ml of water. Add 1 ml of sodium hydroxide (~80 g/l) TS and 4 ml of carbon disulfide R, and shake for 2 minutes. Separate the aqueous layer. Centrifuge the lower layer if necessary, and filter through a dry filter, collecting the filtrate in a small flask provided with a glass stopper. Carry out the examination of the filtered solution using carbon disulfide R as the blank as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diethylcarbamazine dihydrogen citrate RS treated similarly or with the *reference spectrum* of diethylcarbamazine base.

B. Dissolve 0.5 g in 10 ml of water, add 10 ml of sodium hydroxide (1 mol/l)VS, and extract with 4 successive quantities, each of 5 ml of chloroform R. Retain the aqueous layer for test C. Wash the combined chloroform extracts with water, filter through a plug of cotton wool, and evaporate the chloroform. Add 1 ml of ethyl iodide R to the residue, and heat gently under a reflux condenser for 5 minutes. Cool, separate the viscous yellow oil, and dissolve it in ethanol (~750 g/l) TS. Add, with continuous stirring, sufficient ether R to precipitate the quaternary ammonium salt, and filter. Dissolve the precipitate in ethanol (~750 g/l) TS, reprecipitate with ether R, and dry at 105 °C; melting temperature, about 152 °C (1-diethylcarbamoyl-4-methylpiperazine ethiodide).

C. The aqueous layer from test B yields reaction B described under 2.1 General identification tests as characteristic of citrates.

D. Melting temperature, after drying at 80 °C, about 137 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

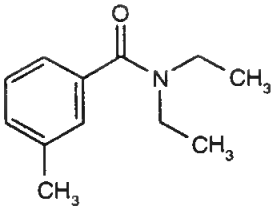
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 10 mg/g.

pH value. pH of a 30 mg/ml solution, 3.5–4.5.

***N*-Methylpiperazine.** Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 6 volumes of ethanol (~750 g/l) TS, 3 volumes of glacial acetic acid R and 1 volume of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.050 mg of *N*-methylpiperazine R per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a mixture of 3 volumes of platinic chloride (60 g/l) TS, 97 volumes of water and 100 volumes of potassium iodide (60 g/l) TS, and examine the chromatogram in daylight. The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 39.14 mg of $C_{10}H_{21}N_3O,C_6H_8O_7$.

DIETHYLTOLUAMIDUM
DIETHYLTOLUAMIDE



$C_{12}H_{17}NO$

Relative molecular mass. 191.3

Chemical name. *N,N*-Diethyl-*m*-toluamide; *N,N*-diethyl-3-methylbenzamide; CAS Reg. No. 134-62-3.

Description. Colourless or faintly yellow liquid.

Solubility. Practically immiscible in water and glycerol R; miscible with ethanol (~750 g/l) TS and ether R.

Category. Insect repellent.

Storage. Diethyltoluamide should be kept in a tightly closed container.

Additional information. *CAUTION*: Diethyltoluamide is an irritant to eyes and mucous membranes.

Requirements

Diethyltoluamide contains not less than **97.0%** and not more than **103.0%** of $C_{12}H_{17}NO$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diethyltoluamide RS or with the *reference spectrum* of diethyltoluamide.

B. Refractive index, $n_D^{20} = 1.520 - 1.524$.

C. To about 2 ml, add 25 ml of hydrochloric acid (~250 g/l) TS and heat under a reflux condenser for 1 hour. Neutralize the solution with sodium hydroxide (~200 g/l) TS, cool, and extract with three quantities, each of 30 ml, of ether R. (Keep the aqueous layer for test D.) Carefully evaporate the ether layer to dryness on a water-bath, and dissolve the residue in 5 ml of sodium nitrite (100 g/l) TS. Allow to stand at 5 °C for 10 minutes, add 10 ml of water, and extract with 20 ml of ether R. Evaporate the ether layer and add to the residue 1.0 g of phenol R. Cool and add about 1 ml of sulfuric acid (~1760 g/l) TS; an intense green solution is produced. Pour the mixture into water; the colour turns to red. Add sodium hydroxide (~80 g/l) TS; the colour changes to green.

D. Acidify the aqueous layer obtained in test C with hydrochloric acid (~70 g/l) TS, extract with two quantities, each of 20 ml of ether R, and carefully evaporate the ether layer. Dry the residue at 60 °C; the melting temperature of the residue is about 108 °C.

Mass density. ρ_{20} = 0.996–1.002.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

Acidity. Dissolve 10.0 g in 50 ml of neutralized ethanol TS, titrate with sodium hydroxide (0.01 mol/l) VS using phenolphthalein/ethanol TS as indicator; not more than 4.0 ml of sodium hydroxide (0.01 mol/l) VS is required to obtain the midpoint of the indicator (pink).

Assay. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.3 g, accurately weighed, and 7 ml of nitrogen-free sulfuric acid (~1760 g/l) TS, and proceed with the distillation. Titrate with sulfuric acid (0.05 mol/l) VS using methyl red/ethanol TS as indicator. Repeat the procedure without the Diethyltoluamide being examined and make any necessary corrections.

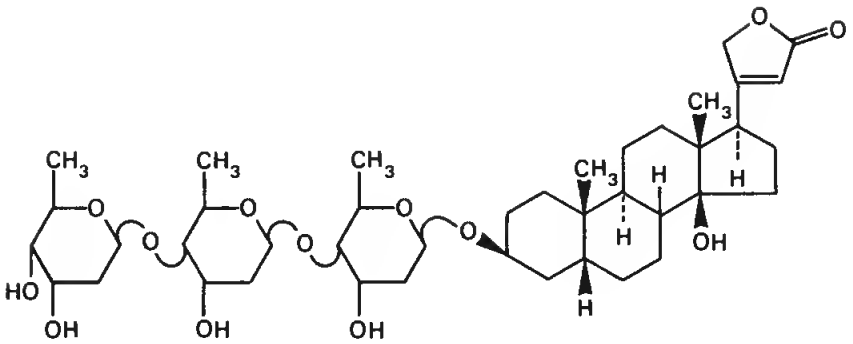
Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 19.13 mg of $C_{12}H_{17}NO$.

DIGITOXINUM
DIGITOXIN

Molecular formula. $C_{41}H_{64}O_{13}$

Relative molecular mass. 765.0

Graphic formula.



Chemical name. 3β-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1β4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)-oxy]-14-hydroxy-5β-card-20(22)-enolide; CAS Reg. No. 71-63-6.

Description. A white or almost white, microcrystalline powder; odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS.

Category. Cardiotonic.

Storage. Digitoxin should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Digitoxin is extremely poisonous and should be handled with care.

Requirements

Definition. Digitoxin contains not less than 95.0% and not more than 105.0% of $C_{41}H_{64}O_{13}$, calculated with reference to the dried substance.

Identity tests

- Either tests A, B and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from digitoxin RS or with the *reference spectrum* of digitoxin.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 15 cm, remove the plate from the chromatographic chamber and allow to stand for at least 5 minutes. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 50 volumes of xylene R, 50 volumes of ethylmethylketone R and 4 volumes of formamide R. Apply separately to the plate 3 µl of each of 2 solutions (A) of the test substance, and (B) of digitoxin RS, each prepared by dissolving 50 mg in a mixture of equal volumes of chloroform R and methanol R to produce 10 ml and then diluting 1 ml to 5 ml with methanol R. Develop the plate for a distance of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry at 115 °C for 20 minutes, cool, spray with a mixture of 15 volumes of a solution of 25 g of trichloroacetic acid R in 100 ml of ethanol (~750 g/l) TS and 1 volume of a freshly prepared 30 mg/ml solution of tosylchloramide sodium R, and then heat the plate at 115 °C for 5 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 1 mg in 1 ml of ethanol (~750 g/l) TS by heating gently. Cool the solution and add 1 ml of dinitrobenzene/ethanol TS and 1 ml of potassium hydroxide (1 mol/l) VS; a violet colour develops and then fades.

D. Dissolve 1 mg in 2 ml of a solution prepared by mixing 0.5 ml of ferric chloride (25 g/l) TS and 100 ml of glacial acetic acid R; cautiously add 1 ml of sulfuric acid (~1760 g/l) TS to form a lower layer; a brown ring, but no red colour, is produced at the junction of the two liquids, and after some time the acetic acid layer acquires a blue colour (distinction from allied glycosides).

Specific optical rotation. Use a 10 mg/ml solution in chloroform R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +16.5$ to $+18.5°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Gitoxin. Dissolve about 5 mg, accurately weighed, in 1 ml of methanol R and dilute to 25 ml with a mixture of equal volumes of hydrochloric acid (~250 g/l) TS and glycerol R. Allow to stand for 1 hour. The absorbance of a 1-cm layer of this solution at 352 nm, when measured against a solvent cell containing a mixture of equal volumes of hydrochloric acid (~250 g/l) TS and glycerol R, is not more than 0.28 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer); the gitoxin content is about 50 mg/g.

Assay. Dissolve about 0.05 g, accurately weighed, in sufficient methanol R to produce 25 ml; dilute 5.0 ml of this solution to 100 ml with methanol R. Place 5.0 ml of the dilute solution to be tested in a 25-ml volumetric flask, add 15 ml of alkaline trinitrophenol TS, and dilute to 25 ml with methanol R. Set aside for 30 minutes, protected from light, and measure the absorbance in a 1-cm layer at the maximum at about 490 nm against a solvent cell containing a solution prepared by diluting 15 ml of alkaline trinitrophenol TS to 25 ml with methanol R. Calculate the amount of $C_{41}H_{64}O_{13}$ in the substance being tested by comparison with digitoxin RS, similarly and concurrently examined.

Additional requirements for Digitoxin for parenteral use

Complies with the monograph for "Parenteral preparations".

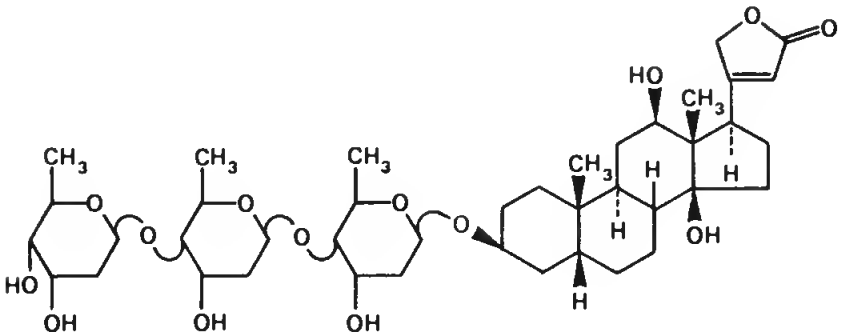
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 111.0 IU of endotoxin RS per mg.

DIGOXINUM
DIGOXIN

Molecular formula. $C_{41}H_{64}O_{14}$

Relative molecular mass. 781.0

Graphic formula.



Chemical name. 3β-[(*O*-2,6-Dideoxy-β-D-*ribo*-hexopyranosyl)-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1β4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)-oxy]-12β,14-dihydroxy-5β-card-20(22)-enolide; CAS Reg. No. 20830-75-5.

Description. Colourless crystals or a white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water and ether R; freely soluble in pyridine R; slightly soluble in ethanol (~750 g/l) TS.

Category. Cardiotonic.

Storage. Digoxin should be kept in a well-closed container, protected from light.

Additional information. CAUTION: Digoxin is extremely poisonous and should be handled with care.

Requirements

Definition. Digoxin contains not less than 95.0% and not more than 103.0% of $C_{41}H_{64}O_{14}$, calculated with reference to the dried substance.

Identity tests

- Either tests A, B and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from digoxin RS or with the *reference spectrum* of digoxin.

B. See the test described below under "Related substances". In daylight the principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 1 mg in 1 ml of ethanol (~750 g/l) TS by heating gently. Cool the solution, add 1 ml of dinitrobenzene/ethanol TS and 1 ml of potassium hydroxide (1 mol/l) VS; a violet colour develops and then fades.

D. Dissolve 1 mg in 2 ml of a solution prepared by mixing 0.5 ml of ferric chloride (25 g/l) TS and 100 ml of glacial acetic acid R; cautiously add 1 ml of sulfuric acid (~1760 g/l) TS to form a lower layer; a brown ring, but no red colour, is produced at the junction of the two liquids, and after some time the acetic acid layer acquires a blue colour (distinction from allied glycosides).

Specific optical rotation. Use a 0.10 g/ml solution in pyridine R; $[\alpha]_{546\,nm}^{20\,°C}$ = +13.6 to +14.2 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 10 mg/g.

Gitoxin. Dissolve about 5 mg, accurately weighed, in 1 ml of ethanol (~675 g/l) TS by heating gently, and dilute to 25 ml with a mixture of equal volumes of hydrochloric acid (~250 g/l) TS and glycerol R. Allow to stand for 1 hour. The absorbance of a 1-cm layer of this solution at 352 nm, when measured against a solvent cell containing a mixture of equal volumes of hydrochloric acid (~250 g/l) TS and glycerol R, is not more than 0.22 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer); about 40 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 15 cm, remove the plate from the chromatographic chamber and allow to stand for at least 5 minutes. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 50 volumes of xylene R, 50 volumes of ethylmethylketone R, and 4 volumes of formamide R. Apply separately to the plate 1 µl of each of 3 solutions in a mixture of equal volumes of methanol R and chloroform R containing (A) 5.0 mg of the test substance per ml, (B) 5.0 mg of digoxin RS per ml and (C) 0.25 mg of digitoxin RS per ml. Develop the plate for a distance of 12 cm. After removal of the plate from the chromatographic chamber, allow it to dry at 115 °C for 20 minutes, cool, spray with a mixture of 15 volumes of a solution of 25 g of trichloroacetic acid R in 100 ml of ethanol (~750 g/l) TS and 1 volume of a freshly prepared 30 mg/ml solution of tosylchloramide sodium R, and then heat the plate at 115 °C for 5 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). Any spot obtained with solution A in ultraviolet light, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Dissolve about 0.05 g, accurately weighed, in sufficient methanol R to produce 25 ml; dilute 5.0 ml of this solution to 100 ml with methanol R. Place 5.0 ml of the dilute solution to be tested in a 25-ml volumetric flask, add 15 ml of alkaline trinitrophenol TS, and dilute to 25 ml with methanol R. Set aside for 30 minutes protected from light, and measure the absorbance in a 1-cm layer at the maximum of about 490 nm against a solvent cell containing a solution prepared by diluting 15 ml of alkaline trinitrophenol TS to 25 ml with methanol

R. Calculate the amount of $C_{41}H_{64}O_{14}$ in the substance being tested by comparison with digoxin RS, similarly and concurrently examined.

Additional requirements for Digoxin for parenteral use

Complies with the monograph for "Parenteral preparations".

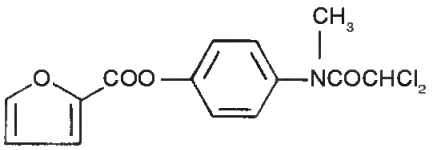
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 200.0 IU of endotoxin RS per mg.

DILOXANIDI FUROAS
DILOXANIDE FUROATE

Molecular formula. $C_{14}H_{11}Cl_2NO_4$

Relative molecular mass. 328.2

Graphic formula.



Chemical name. 2,2-Dichloro-4′-hydroxy-*N*-methylacetanilide 2-furoate (ester); 4-[(dichloroacetyl)methylamino]phenyl 2-furancarboxylate; 2,2-dichloro-*N*-(4-hydroxyphenyl)-*N*-methylacetamide 2-furoate; CAS Reg. No. 3736-81-0.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; soluble in 100 parts of ethanol (~750 g/l) TS and in 130 parts of ether R.

Category. Antiamoebic drug.

Storage. Diloxanide furoate should be kept in a well-closed container, protected from light.

Requirements

Definition. Diloxanide furoate contains not less than 98.0% and not more than 102.0% of $C_{14}H_{11}Cl_2NO_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diloxanide furoate RS or with the *reference spectrum* of diloxanide furoate.

B. The absorption spectrum of a 7.0 µg/ml solution in ethanol (~750 g/l) TS, when observed between 240 nm and 350 nm, exhibits a maximum at about 258 nm; the absorbance of a 1-cm layer at this wavelength is about 0.49.

C. Carry out the combustion as described under 2.4 Oxygen flask method, using 20 mg of the test substance and 10 ml of sodium hydroxide (1 mol/l) VS as the absorbing liquid. When the process is complete, acidify with nitric acid (~130 g/l) TS; the solution yields reaction A, described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 114–116 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Free acidity. Shake 3.0 g with 50 ml of carbon-dioxide-free water R, filter and wash the residue with 3 quantities, each of 20 ml of carbon-dioxide-free water R. Titrate the combined filtrate and washings with sodium hydroxide (0.1 mol/l) VS, phenolphthalein/ethanol TS being used as indicator; not more than 1.3 ml is required.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 24 volumes of dichloromethane R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in chloroform R containing (A) 0.10 g of the test substance per ml and (B) 2.5 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

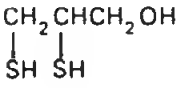
Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of anhydrous pyridine R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 32.82 mg of $C_{14}H_{11}Cl_2NO_4$.

DIMERCAPROLUM
DIMERCAPROL

Molecular formula. $C_3H_8OS_2$

Relative molecular mass. 124.2

Graphic formula.



Chemical name. 2,3-Dimercapto-1-propanol; CAS Reg. No. 59-52-9.

Description. A clear, colourless or slightly yellow liquid, with an unpleasant, mercaptan-like odour.

Miscibility. Miscible with 20 parts of water; miscible with ethanol (~750 g/l) TS and methanol R.

Category. Antidote for arsenic, gold, and mercury poisoning.

Storage. Dimercaprol should be kept in a small, well-filled and tightly closed container, protected from light, and stored at a temperature not exceeding 5 °C.

Requirements

Definition. Dimercaprol contains not less than 98.5% w/w and not more than 101.5% w/w of $C_3H_8OS_2$.

Identity tests

A. Mix 0.05 ml of cobalt(II) chloride (30 g/l) TS with 5 ml of water and add 0.05 ml of the test liquid; a yellow-brown colour is produced.

B. Dissolve 0.1 ml in 4 ml of water and add a few drops of lead acetate (80 g/l) TS; a yellow precipitate is formed.

Refractive index. $n_D^{20} = 1.568 - 1.574$.

Relative density. $d_{20}^{20} = 1.239 - 1.259$.

Halides. Dissolve 2.0 g in 25 ml of potassium hydroxide/ethanol TS1 and heat under a reflux condenser for 2 hours. Evaporate the ethanol in a current of warm air, add 20 ml of water, and cool. Add a mixture of 10 ml of hydrogen peroxide

(~330 g/l) TS and 40 ml of water, boil gently for 10 minutes, cool, and filter rapidly. Add 10 ml of nitric acid (~130 g/l) TS and 5 ml of silver nitrate (0.1 mol/l) VS and titrate with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the operation without the test liquid being examined. The difference between the titrations does not exceed 1.0 ml.

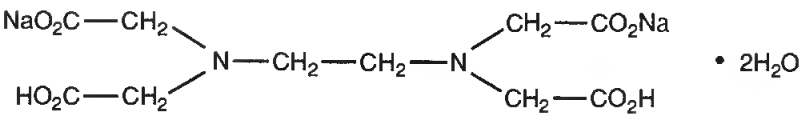
pH value. pH of a 0.5 g/ml solution in carbon-dioxide-free water R, 4.6–6.8.

Assay. Dissolve about 0.12 g, accurately weighed, in 20 ml of hydrochloric acid (0.1 mol/l) VS and titrate rapidly with iodine (0.05 mol/l) VS, using starch TS as indicator. Repeat the operation without the test liquid being examined and make any necessary corrections. Each ml of iodine (0.05 mol/l) VS is equivalent to 6.211 mg of $C_3H_8OS_2$.

Additional requirement for Dimercaprol for parenteral use

Complies with the monograph for "Parenteral preparations".

DINATRII EDETAS
DISODIUM EDETATE



$C_{10}H_{14}N_2Na_2O_8,2H_2O$

Relative molecular mass. 372.2

Chemical name. Disodium dihydrogen (ethylenedinitrilo)tetraacetate dihydrate; *N*,*N*′-1,2-ethanediylbis[*N*-(carboxymethyl)glycine] disodium salt, dihydrate; CAS Reg. No. 6381-92-6.

Other name. Edetate disodium.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Stabilizer; chelating agent.

Storage. Disodium edetate should be kept in a well-closed container.

Additional information. Solutions of disodium edetate should not come into contact with metal.

Requirements

Disodium edetate contains not less than **98.5%** and not more than the equivalent of **101.0%** of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from disodium edetate R or with the *reference spectrum* of disodium edetate.

B. To 3 drops of ferric chloride (25 g/l) TS add 3 drops of ammonium thiocyanate (75 g/l) TS; to the deep red solution produced add 0.05 g of Disodium edetate; the colour is discharged, leaving a yellowish solution. (Keep this solution for test D.)

C. Dissolve 2 g in 25 ml of water, add 2 ml of lead nitrate (100 g/l) TS, shake, and add 6 ml of potassium iodide (80 g/l) TS; no yellow precipitate is observed.

D. To the solution from test B, add ammonia (~100 g/l) TS, drop by drop, until an alkaline reaction is obtained with pH-indicator paper R. Add 5 ml of ammonium oxalate (25 g/l) TS; no precipitate is produced (distinction from sodium calcium edetate).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

pH value. pH of a 0.05 g/ml solution, 4.0–5.5.

Assay. Dissolve 0.5 g, accurately weighed, in sufficient water to produce 300 ml. Add 2 g of methenamine R and 2 ml of hydrochloric acid (~70 g/l) TS. Titrate with lead nitrate (0.1 mol/l) VS to which 50 mg of xylenol orange indicator mixture R has been added.

Each ml of lead nitrate (0.1 mol/l) VS is equivalent to 37.22 mg of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

DINITROGENII OXIDUM
DINITROGEN OXIDE

N_2O

Relative molecular mass. 44.01

Chemical name. Nitrous oxide; CAS Reg. No. 10024-97-2.

Other name. Nitrous oxide.

Description. A colourless gas; odourless.

Solubility. One volume dissolves in about 1.5 volumes of water at a pressure of 101.3 kPa and a temperature of 20 °C.

Category. Inhalational anaesthetic gas.

Storage. Dinitrogen oxide should be kept as compressed gas or liquid at very low temperatures, in appropriate containers complying with the safety regulations of the national authority. Valves or taps should not be lubricated with oil or grease.

Labelling. An ISO standard[1] requires that cylinders containing Dinitrogen oxide intended for medical use should bear the name of the contents in legible and permanent characters and, preferably, also the molecular formula N_2O.

Additional information. In the analysis of medicinal gases certain tests are not intended for hospital pharmacists. They are applicable solely by laboratories equipped with specialized apparatus.

Requirements

Dinitrogen oxide contains not less than **98.0% v/v** of N_2O in the gaseous phase, when sampled at 15 °C.

Note: If the analysis is performed on a cylinder, keep the cylinder of the gas to be examined at room temperature for at least 6 hours before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

[1] *International Standard 32. Gas cylinders for medical use – marking for identification content.* International Organization for Standardization, Switzerland, 1977.

The test for carbon monoxide should be carried out on the first portion of gas drawn from the container and the tests for nitrogen monoxide and nitrogen dioxide immediately thereafter.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of dinitrogen oxide.

B. Place a glowing splinter of wood into the gas; the splinter bursts into flame.

C. Shake the gas with alkaline pyrogallol TS; it is not absorbed and the solution does not become brown (distinction from oxygen).

D. Mix the gas with an equal volume of nitrogen monoxide R; no red fumes are produced (distinction from oxygen).

Carbon monoxide

- Either test A, test B, or test C may be applied.

- The tests should be carried out on the first portion of gas released from the container.

A. The apparatus (Fig. 6) consists of the following parts connected in series:

— a U-tube (U1) containing desiccant silica gel R impregnated with chromium trioxide R;

— a wash bottle (F1) containing 100 ml of potassium hydroxide (~400 g/l) TS;

— a U-tube (U2) containing pellets of potassium hydroxide R;

— a U-tube (U3) containing phosphorus pentoxide R dispersed on previously granulated, fused pumice;

— a U-tube (U4) containing 30 g of recrystallized iodine pentoxide R in granules, previously dried at 200 °C and kept at a temperature of 120 °C (T) during the test. The iodine pentoxide is packed in the tube in 1-cm columns separated by 1-cm columns of glass wool to give an effective length of 5 cm;

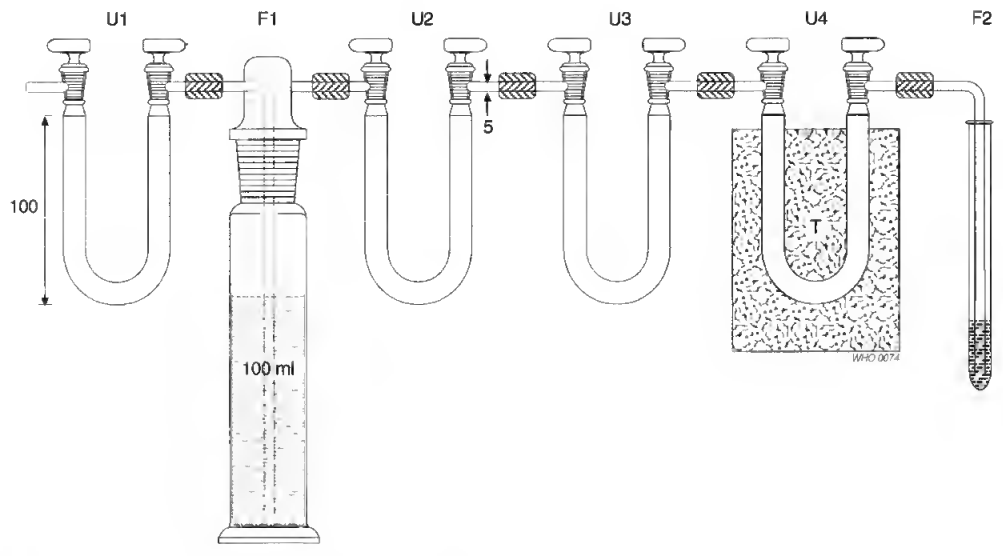


Figure 6. Apparatus for the determination of carbon monoxide in medicinal gases
Measurements in mm.
Reproduced with the permission of the European Pharmacopoeia Commission, European Directorate for the Quality of Medicines, Council of Europe.

— a reaction tube (F2) containing 2.0 ml of potassium iodide (160 g/l) TS and 0.15 ml of starch TS.

Flush the apparatus with 5.0 litres of argon R. If necessary, discharge the blue colour in tube F2 containing potassium iodide (160 g/l) TS by adding a sufficient volume of freshly prepared sodium thiosulfate (0.002 mol/l) VS. Continue flushing with argon R until not more than 0.045 ml of sodium thiosulfate (0.002 mol/l) VS is required after the passage of 5.0 litres of argon R. Pass 5.0 litres of the test gas from the container through the apparatus. Flush the last traces of liberated iodine into the reaction tube by passing 1.0 litre of argon R through the apparatus. Titrate the liberated iodine with sodium thiosulfate (0.002 mol/l) VS. Repeat the procedure using 5.0 litres of argon R.

The difference between the volumes of sodium thiosulfate (0.002 mol/l) VS used in the titrations is not more than 0.25 ml (5 µl/l).

B. Carry out the test as described under 1.14.5 Gas chromatography, using a stainless steel column (2 m × 4 mm) packed with a 0.5-nm molecular sieve (e.g. ×13, obtainable from a commercial source). Maintain the column at 80 °C, and the injection port and the detector at room temperature. Use helium R as the carrier gas at a flow rate of 60 ml per minute, and a helium ionization detector.

Use the following gases: (1) the test gas; and (2) a mixture containing 5 µl of carbon monoxide R in 1 litre of dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to produce a peak response for carbon monoxide obtained with the reference gas (2) that gives a height of not less than 5% on the recorder.

Measure the areas of the peak responses obtained in the chromatograms from injections 1 and 2 and calculate the content of carbon monoxide in the test gas (1) by comparing with the peak response for carbon monoxide obtained from the reference gas (2); not more than 5 µl/l.

C. Determine the content using a carbon monoxide detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the carbon monoxide detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 5 µl/l.

Note: For the following tests – Nitrogen monoxide and nitrogen dioxide, Carbon dioxide Test A, Halogens and hydrogen sulfide, and Acidity and alkalinity – pass the gas to be tested through the appropriate reagent contained in a hermetically closed flat-bottomed glass cylinder, with dimensions such that 50 ml of liquid reaches a height of 12–14 cm, that is fitted with (a) a delivery tube terminated by a capillary 1 mm in internal diameter and placed within 2 mm of the bottom of the cylinder; and (b) an outlet tube.

Prepare the reference solutions in identical cylinders.

Nitrogen monoxide and nitrogen dioxide

- Either test A or test B may be applied.

- This test should be performed after release of the 5.0 litres of gas as described above under "Carbon monoxide, test A".

A. Pass the test gas through two of the cylinders connected in series as described above under "Carbon monoxide, test A". To obtain the liquid phase invert the gas cylinder; the liquid vaporizes on leaving the valve.

To 50 ml of water add 1.2 ml of sulfuric acid (~1760 g/l) TS and dilute with sufficient water to produce 100 ml. To 15 ml of this solution add 375 mg of potassium permanganate R, mix, and transfer to the first cylinder (*solution A*).

Dissolve 1 g of sulfanilic acid R in a mixture of 180 ml of water and 10 ml of glacial acetic acid R (solution 1). Separately dissolve 0.2 g of *N*-(1-naphthyl)ethylenediamine hydrochloride R in a mixture of 4 ml of glacial acetic acid R and 5 ml of water, heat gently, and dilute to 200 ml with water (solution 2). Mix 1 volume of solution 2 with 9 volumes of solution 1 and transfer 20 ml of this mixture to the second cylinder (*solution B*).

Connect the outlet tube of the first cylinder to the delivery tube of the second cylinder containing solution B. Pass 2.5 litres of the test gas through the reagents at a rate of 15.0 litres per hour.

Prepare a reference solution by adding 0.25 ml of a solution containing 61.6 µg/ml of sodium nitrite R in water to 20 ml of solution B as prepared above. Allow the test solution and reference solution to stand for 10 minutes.

Examine the gaseous and the liquid phases separately.

For both gaseous and liquid phases, any red colour produced from the solution of the test gas is not more intense than that from the reference solution (2 µl/l of $NO + NO_2$).

B. Determine the content using a nitrogen monoxide and nitrogen dioxide detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the nitrogen monoxide and nitrogen dioxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 2 µl/l.

Carbon dioxide

- Either test A, test B, or test C may be applied.

A. Pass 1.0 litre of the test gas through 50 ml of a clear solution of barium hydroxide (0.15 mol/l) VS. Similarly prepare a reference solution by adding

1.0 ml of a 1.1 mg/ml solution of sodium hydrogen carbonate R in carbon-dioxide-free water R to 50 ml of barium hydroxide (0.15 mol/l) VS.

Any turbidity in the solution after the passage of the test gas is not more intense than that of the reference solution (300 µl/l).

B. Carry out the test as described under 1.14.5 Gas chromatography, using a stainless steel column (3.5 m × 2 mm) packed with ethylvinyl-benzenedivinylbenzene copolymer. Maintain the column at 40 °C and the detector at 90 °C. Use helium R as the carrier gas at a flow rate of 15 ml per minute, and a thermal conductivity detector.

Use the following gases: (1) the test gas; and (2) a mixture containing 300 µg of carbon dioxide R in 1 litre of dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to obtain a peak response for carbon dioxide obtained with the reference gas (2) of a height of not less than 35% on the recorder.

Measure the areas of the peak responses obtained in the chromatograms from the injections of gases 1 and 2 and calculate the content of carbon dioxide in the test gas (1) by comparing with the peak response for carbon dioxide obtained from the reference gas (2); not more than 300 µl of CO_2 per litre.

C. Determine the content using a carbon dioxide detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the carbon dioxide detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 300 µl/l.

Halogens and hydrogen sulfide. Pass 20.0 litres of the test gas through a mixture of 1 ml of silver nitrate (40 g/l) TS and 49 ml of water at a flow rate not exceeding 15 litres per hour.

Prepare the reference solution as follows: to 1.0 ml of silver nitrate (40 g/l) TS add 40 ml of chloride standard (5 µg/ml) TS and 0.15 ml of nitric acid (~130 g/l) TS, dilute to 50 ml with water, and allow to stand protected from light for 5

minutes. For the blank solution, repeat the procedure passing the test gas through 50 ml of water.

Compare a 100-mm layer of the solution as described under 1.11 Colour of liquids.

The solution of the test gas does not darken when compared with the blank. Any opalescence is not more intense than that of the reference solution (10 µg Cl per litre of dinitrogen oxide).

Water

- Either test A or test B may be applied.

A. The apparatus consists of either an electrolytic hygrometer as described below, an appropriate humidity detector tube, or a capacity hygrometer.

   The measuring cell consists of a thin film of phosphoric anhydride placed between two coiled platinum wires which act as electrodes. The water vapour in Dinitrogen oxide is absorbed by the phosphoric anhydride to form phosphoric acid which acts as an electrical conductor.

   Before introducing the test gas into the device, allow the gas to stabilize at room temperature and make sure that the temperature is constant throughout the apparatus. Apply a continuous voltage across the electrodes to produce electrolysis of the water and regeneration of phosphoric anhydride. Measure the resulting electric current, which is proportional to the water content in the test gas. (This is a self-calibrating system that obeys Faraday's law.)

   Calculate the content of water; not more than 60 µg/l.

B. Determine the content using a water vapour detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

   The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the water vapour detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 60 µl/l.

Acidity and alkalinity. Pass 2.0 litres of the test gas through a mixture of 0.10 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

For *reference solution 1*, use 50 ml of carbon-dioxide-free water R. For *reference solution 2*, use a mixture of 0.20 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

To each solution add 0.1 ml of methyl red/ethanol TS; the intensity of the colour in the test gas solution is between that of reference solutions 1 and 2.

Assay. Determine as described under 1.14.5 Gas chromatography, using a stainless steel column (2 m × 2 mm) packed with silica gel for chromatography R (250—355 µm). Maintain the column at 60 °C and the detector at 130 °C. Use helium R as the carrier gas at a flow rate of 50 ml per minute, and a thermal conductivity detector.

Use the following gases: (1) the test gas; and (2) dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to produce a peak response for dinitrogen oxide obtained with reference gas (2) that gives a height of not less than 35% on the recorder.

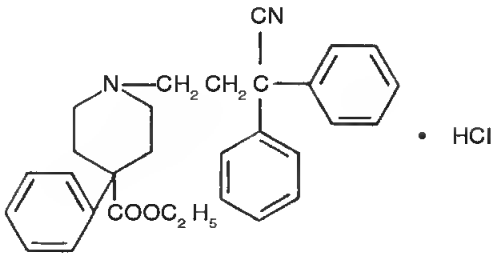
Measure the areas of the peak responses obtained in the chromatograms from the injections of gases (1) and (2), and calculate the percentage content of dinitrogen oxide.

DIPHENOXYLATI HYDROCHLORIDUM
DIPHENOXYLATE HYDROCHLORIDE

Molecular formula. $C_{30}H_{32}N_2O_2,HCl$

Relative molecular mass. 489.1

Graphic formula.



Chemical name. Ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisonipecotate monohydrochloride; ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenyl-4-piperidinecarboxylate monohydrochloride; CAS Reg. No. 3810-80-8.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Sparingly soluble in water, acetone R and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antidiarrhoeal drug.

Storage. Diphenoxylate hydrochloride should be kept in a well-closed container.

Requirements

Definition. Diphenoxylate hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{30}H_{32}N_2O_2$,HCl, calculated with reference to the dried substance.

Identity tests

- Either tests A and E or tests B, C, D and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diphenoxylate hydrochloride RS or with the *reference spectrum* of diphenoxylate hydrochloride.

B. The absorption spectrum of a 0.50 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, when observed between 230 nm and 350 nm, exhibits maxima at about 252 nm, 258 nm, and 265 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.55, 0.65 and 0.50, respectively.

C. Dissolve 25 mg in 5 ml of water and add 0.1 ml of potassio-mercuric iodide TS; a cream-coloured precipitate is produced.

D. Melting temperature, about 223 °C.

E. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

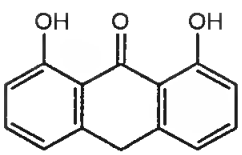
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 92 volumes of chloroform R, 3 volumes of methanol R, and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 50 mg of the test substance per ml and (B) 0.50 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of iodine, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 40 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 48.91 mg of $C_{30}H_{32}N_2O_2$,HCl.

DITHRANOLUM
DITHRANOL



$C_{14}H_{10}O_3$

Relative molecular mass. 226.2

Chemical name. 1,8,9-Anthratriol; CAS Reg. No. 1143-38-0.

Other name. Anthralin.

Description. A yellow or brownish yellow, crystalline powder.

Solubility. Practically insoluble in water; soluble in dichloromethane R; sparingly soluble in acetone R; slightly soluble in ethanol (~750 g/l) TS and ether R.

Category. Keratolytic agent.

Storage. Dithranol should be kept in a tightly closed container, protected from light.

Requirements

Dithranol contains not less than **98.5%** and not more than **101.0%** of $C_{14}H_{10}O_3$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dithranol RS or with the *reference spectrum* of dithranol.

B. The absorption spectrum of a 10 µg/ml solution in dichloromethane R, when observed between 250 nm and 450 nm, exhibits 3 maxima at about 256 nm, 288 nm, and 356 nm. The absorbance of a 1-cm layer at the maximum wavelength at 356 nm is about 0.46 and at 288 nm about 0.49.

C. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of equal volumes of hexane R and dichloromethane R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in dichloromethane R containing (A) 1.0 mg of Dithranol per ml, (B) 1.0 mg of dithranol RS, and for solution (C) dissolve 5 mg of dantron R in 5 ml of solution B. After removing the plate from the chromatographic chamber, allow it to dry in air. Place the plate in a chamber saturated with ammonia vapour until the spots appear. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

D. Melting temperature, about 180 °C.

Chlorides. Dissolve 2.5 g in a mixture of 2.0 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.1 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5 mg/g.

pH value. Shake 1.5 g with 30 ml of carbon-dioxide-free water R for 1 minute and filter; pH of the filtrate, 6.0–7.6.

Related substances

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of porous silica (5 µm). As the mobile phase, use a mixture of 82 volumes of hexane R, 5 volumes of dichloromethane R, and 1 volume of glacial acetic acid R.

Prepare the following solutions. For solution (A) dissolve 0.20 g of Dithranol in 20 ml of dichloromethane R, add 1.0 ml of glacial acetic acid R, and dilute to 100 ml with hexane R. For solution (B) dissolve 10.0 mg of each of anthrone R, dantron R, 9,9′-bisanthracene-10,10′(9*H*,9′*H*)- dione RS, and dithranol RS in dichloromethane R, and dilute to 10.0 ml with the same solvent. To 1.0 ml of this solution add 19 ml of dichloromethane R and 1.0 ml of glacial acetic acid R, and dilute to 50 ml with hexane R.

Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Inject 20 µl each of solutions A and B. Continue the chromatography for 1.5 times the retention time of the peak due to 9,9′-bisanthracene-10,10′(9*H*,9′*H*)-dione obtained with solution B. Adjust the sensitivity of the system so that the height of the peak due to dithranol in the chromatogram obtained with solution B is about 70% of the full scale of the recorder. The peaks are eluted in the following order: dithranol, dantron, anthrone and 9,9′-bisanthracene-10,10′(9*H*,9′*H*)-dione. The test is not valid unless, in the chromatogram obtained with solution B, the resolution between the peaks due to dithranol and dantron is greater than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to anthrone, dantron or 9,9′-bisanthracene-10,10′(9*H*,9′*H*)-dione is not greater than that of the corresponding peak in the chromatogram obtained with solution B (1.0%). The area of any peak,

other than the principal peak and any peaks due to anthrone, dantron or 9,9′-bisanthracene-10,10′(9*H*,9′*H*)-dione, is not greater than that of the peak due to dithranol in the chromatogram obtained with solution B (1.0%).

B. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (20 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 60 volumes of water, 40 volumes of tetrahydrofuran R, and 2.5 volumes of glacial acetic acid R.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Dithranol per ml; and for solution (B) dissolve 0.5 mg of 1-hydroxy-9-anthrone RS and 0.5 mg of dithranol RS per ml, and dilute 1.0 ml of this solution to 20 ml with the mobile phase.

Operate with a flow rate of about 0.9 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject 20 µl each of solutions A and B. Continue the chromatography for 3 times the retention time of the peak due to dithranol. The test is not valid unless, in the chromatogram obtained with solution B, the resolution between the peaks due to 1-hydroxy-9-anthrone and dithranol is greater than 2.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to 1-hydroxy-9-anthrone is not greater than that of the corresponding peak in the chromatogram obtained with solution B (2.5%).

The total content of related substances as determined in tests A and B is not more than 3.0%.

Assay. Dissolve about 0.2 g, accurately weighed, in 50 ml of anhydrous pyridine R and titrate under an atmosphere of nitrogen with tetrabutylammonium hydroxide (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method B, determining the end-point potentiometrically.

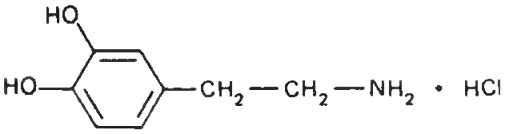
Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 22.62 mg of $C_{14}H_{10}O_3$.

DOPAMINI HYDROCHLORIDUM
DOPAMINE HYDROCHLORIDE

Molecular formula. $C_8H_{11}NO_2,HCl$

Relative molecular mass. 189.6

Graphic formula.



Chemical name. 4-(2-Aminoethyl)pyrocatechol hydrochloride; 4-(2-aminoethyl)-1,2-benzenediol hydrochloride; CAS Reg. No. 62-31-7.

Description. Colourless crystals or a white or almost white, crystalline powder; odourless.

Solubility. Freely soluble in water; soluble in methanol R; practically insoluble in ether R and toluene R.

Category. Cardiovascular drug; sympathomimetic.

Storage. Dopamine hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Dopamine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_8H_{11}NO_2,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dopamine hydrochloride RS or with the *reference spectrum* of dopamine hydrochloride.

B. The absorption spectrum of a 0.020 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a

maximum at about 280 nm and a minimum at about 249 nm; the absorbance of a 1-cm layer at 280 nm is about 0.54.

C. Dissolve 0.05 g in 5 ml of water and add while stirring 10 ml of 4-amino-antipyrine TS2; a red colour is produced.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R1 as the coating substance and a mixture of 13 volumes of chloroform R, 9 volumes of methanol R, and 4 volumes of acetic acid (~300 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 30 mg of the test substance per ml and (B) 0.3 mg of dopamine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry at room temperature for several minutes, then spray it evenly with a freshly prepared mixture of 2 volumes of ferric chloride (50 g/l) TS and 1 volume of potassium ferricyanide (50 g/l) TS. Examine the chromatogram in daylight. Apart from the principal spot, not more than 3 spots are obtained with solution A, and the estimated sum of impurities is not larger than that estimated from the chromatogram obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 140 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 18.96 mg of $C_8H_{11}NO_2$,HCl.

Additional requirements for Dopamine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

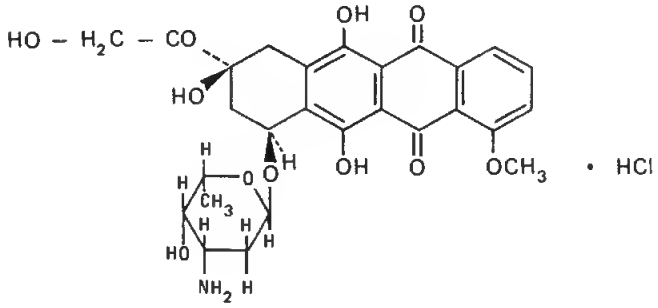
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 16.67 IU of endotoxin RS per mg.

DOXORUBICINI HYDROCHLORIDUM
DOXORUBICIN HYDROCHLORIDE

Molecular formula. $C_{27}H_{29}NO_{11},HCl$

Relative molecular mass. 580.0

Graphic formula.



Chemical name. (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride; (8*S*-*cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride; CAS Reg. No. 25316-40-9.

Description. A red-orange, crystalline powder.

Solubility. Soluble in water and methanol R; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Doxorubicin hydrochloride should be kept in a tightly closed container.

Additional information. Doxorubicin hydrochloride is hygroscopic; it is poisonous. CAUTION: Doxorubicin hydrochloride must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Doxorubicin hydrochloride contains not less than 97.0% and not more than 102.0% of $C_{27}H_{29}NO_{11},HCl$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 20 µg/ml solution in methanol R, when observed between 220 nm and 600 nm, is qualitatively similar to that of a 20 µg/ml solution of doxorubicin hydrochloride RS in methanol R (maxima occur at about 233 nm, 253 nm, 290 nm, 477 nm, 495 nm and 530 nm; minima occur at about 245 nm, 280 nm and 350 nm). The absorbances of the solutions at the respective maxima do not differ from each other by more than 3%. The absorbance of a 1-cm layer at the wavelengths of the main maxima are about 1.32, 0.88, 0.30, 0.46, 0.44 and 0.24, respectively.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

C. Dissolve 2 mg in 2 ml of methanol R, add 2 ml of water and 0.05 ml of sodium hydroxide (~80 g/l) TS; the orange-red colour of the solution turns to blue-violet.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not more than 40 mg/g.

pH value. pH of a 5.0 mg/ml solution, 3.8–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 80 volumes of chloroform R, 20 volumes of methanol R and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in methanol R containing (A) 2.0 mg of the test substance per ml, (B) 2.0 mg of doxorubicin hydrochloride RS per ml, (C) 20 mg of the test substance per ml, and (D) 0.40 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in daylight. Any spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D.

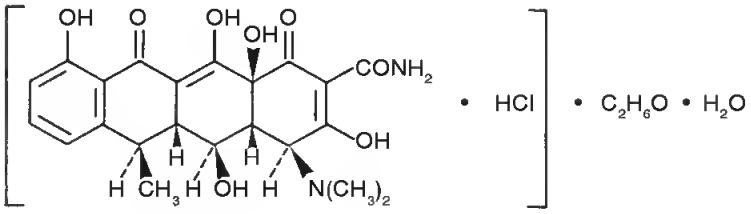
Assay. Dissolve about 20 mg, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 10 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 495 nm. Calculate the amount of $C_{27}H_{29}NO_{11},HCl$ in the substance being tested by comparison with doxorubicin hydrochloride RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.44 ± 0.02.

DOXYCYCLINI HYCLAS
DOXYCYCLINE HYCLATE

Molecular formula. $(C_{22}H_{24}N_2O_8,HCl)_2,C_2H_6O,H_2O$

Relative molecular mass. 1026

Graphic formula.



Chemical name. (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride, compound with ethyl alcohol (2:1), mono-hydrate; [4*S*-(4α,4aα,5α,5aα,6α, 12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride, compound with ethanol (2:1), monohydrate; CAS Reg. No. 24390-14-5.

Description. A yellow, crystalline powder.

Solubility. Soluble in 3 parts of water and in 4 parts of methanol R; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Doxycycline hyclate should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Doxycycline hyclate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Doxycycline hyclate contains not less than 880 International Units of doxycycline per mg, calculated with reference to the anhydrous and ethanol-free substance.

Identity tests

A. Dissolve 5 mg in 2 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced.

B. Dissolve 5 mg in 2.0 ml of water and add 0.05 ml of ferric chloride (~25 g/l) TS; a dark red-brown colour is produced.

C. Dissolve 5 mg in 2.0 ml of water and add 0.25 ml of alkaline potassio-mercuric iodide TS; a fine crystalline, light yellow precipitate is formed.

D. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 4.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1.2 g of the substance; the water content is not less than 14 mg/g and not more than 28 mg/g.

Ethanol. Carry out the test as described under 1.14.5 Gas chromatography, using 3 solutions in water containing (1) a mixture of 0.50 µl of dehydrated ethanol R per ml, (2) 10 mg of the test substance per ml, and (3) a mixture of 10 mg of the test substance per ml with 0.50 µl of the internal standard.

For the procedure use a column 1.5 m long and 4 mm in internal diameter packed with porous polymer beads (particle size 80–100 µm from a commercial source is suitable). Maintain the column at 135 °C, use nitrogen R as the carrier gas and a flame ionization detector.

Calculate the content of ethanol in mg/g, assuming the weight per ml at 20 °C to be 0.790 g; not less than 43 mg/g and not more than 60 mg/g.

pH value. pH of a 10 mg/ml solution, 2.0–3.0.

Absorption in the ultraviolet region. The absorption spectrum of a 10 µg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R exhibits a maximum at about 349 nm. The absorbance of a 1-cm layer at this maximum is not less than 0.28 and not more than 0.31 for the anhydrous and ethanol-free substance.

Light-absorbing impurities. Prepare a 10 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, and measure the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.12 for the anhydrous and ethanol-free substance.

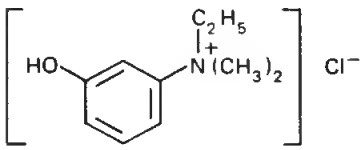
Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus cereus* (NCTC 10320 or ATCC 11778) as the test organism, culture medium Cm10 with a final pH of 6.6, potassium dihydrogen phosphate (13.6 g/l) TS as a buffer, an appropriate concentration of doxycycline (usually between 0.2 and 2.0 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 880 IU of doxycycline per mg, calculated with reference to the anhydrous and ethanol-free substance.

EDROPHONII CHLORIDUM
EDROPHONIUM CHLORIDE

Molecular formula. $C_{10}H_{16}ClNO$

Relative molecular mass. 201.7

Graphic formula.



Chemical name. Ethyl(*m*-hydroxyphenyl)dimethylammonium chloride; *N*-ethyl-3-hydroxy-*N*,*N*-dimethylbenzenaminium chloride; CAS Reg. No. 116-38-1.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 0.5 parts of water and in 5 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Diagnostic agent.

Storage. Edrophonium chloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Edrophonium chloride contains not less than 98.5% and not more than 101.0% of $C_{10}H_{16}ClNO$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.050 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 273 nm; the absorbance of a 1-cm layer at this wavelength is about 0.55.

B. The absorption spectrum of a 10 µg/ml solution in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits maxima at about 240 nm and 294 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.55 and 0.17, respectively.

C. Dissolve 0.05 g in 2 ml of water and add 0.05 ml of ferric chloride (25 g/l) TS; a reddish violet colour is produced.

D. Melting temperature, about 168 °C with decomposition.

E. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 24 hours; it loses not more than 5.0 mg/g.

pH value. pH of a 0.10 g/ml solution, 4.0–5.0.

Dimethylaminophenol. Dissolve 0.1 g in 10 ml of water, add 5 ml of phosphate buffer, pH 8.0, TS, and extract with 2 quantities, each of 20 ml of chloroform R.

Wash the extracts successively with 2 quantities, each of 10 ml of water, and extract with 10 ml of sodium hydroxide (0.1 mol/l) VS. Measure the absorbance of the sodium hydroxide extract using a 1-cm layer at 293 nm; not greater than 0.25.

Assay. Dissolve about 0.20 g, accurately weighed, in 20 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and 0.25 ml of quinaldine red/ethanol TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.17 mg of $C_{10}H_{16}ClNO$.

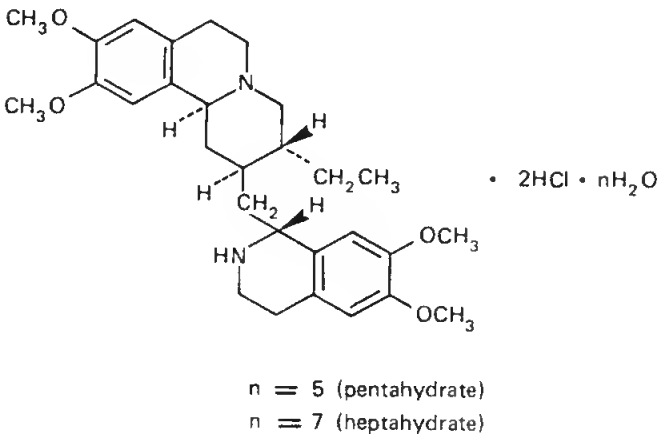
EMETINI HYDROCHLORIDUM
EMETINE HYDROCHLORIDE

Emetine hydrochloride pentahydrate
Emetine hydrochloride heptahydrate

Molecular formula. $C_{29}H_{40}N_2O_4,2HCl,5H_2O$ (pentahydrate); $C_{29}H_{40}N_2O_4$, $2HCl,7H_2O$ (heptahydrate).

Relative molecular mass. 643.6 (pentahydrate); 679.7 (heptahydrate).

Graphic formula.



Chemical name. Emetine dihydrochloride pentahydrate; 6′,7′,10,11-tetramethoxyemetan dihydrochloride pentahydrate; CAS Reg. No. 79300-07-5 (pentahydrate).

Emetine dihydrochloride heptahydrate; 6′,7′,10,11-tetramethoxyemetan dihydrochloride heptahydrate; CAS Reg. No. 79300-08-6 (heptahydrate).

Description. A white or very slightly yellow, crystalline powder; odourless.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS.

Category. Antiamoebic drug.

Storage. Emetine hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container of Emetine hydrochloride should state whether the substance is the pentahydrate or the heptahydrate.

Additional information. Emetine hydrochloride is the hydrochloride of an alkaloid obtained from ipecacuanha or prepared by methylation of cephaëline or by synthesis. Even in the absence of light, Emetine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Emetine hydrochloride contains not less than 98.0% and not more than 101.5% of $C_{29}H_{40}N_2O_4,2HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from emetine hydrochloride RS or with the *reference spectrum* of emetine hydrochloride.

B. See the test described below under "Related alkaloids". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution D.

C. Sprinkle 5 mg of the test substance on the surface of 1 ml of ammonium molybdate/sulfuric acid TS; a bright green colour is produced.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +16 to +19 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; Emetine hydrochloride pentahydrate loses not less than 110 mg/g and not more than 150 mg/g. Emetine hydrochloride heptahydrate loses not less than 150 mg/g and not more than 190 mg/g.

Acidity. Dissolve 0.10 g in 10 ml of carbon-dioxide-free water R and titrate with sodium hydroxide (0.02 mol/l) VS, using methyl red/ethanol TS as indicator; not more than 0.5 ml is required to obtain the midpoint of the indicator (orange).

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 100 volumes of chloroform R, 20 volumes of ethylene glycol monomethyl ether R, 5 volumes of methanol R, 2 volumes of water and 0.5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in a mixture of 1 volume of ammonia (~17 g/l) TS and 99 volumes of methanol R containing (A) 0.50 mg of the test substance per ml, (B) 10 µg of cephaëline hydrochloride R per ml, (C) 5.0 µg of the test substance per ml and (D) 0.50 mg of emetine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with iodine/chloroform TS, heat it at 60 °C for 15 minutes, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A is not more intense than the corresponding spot obtained with solution B. Any other spot obtained with solution A, is not more intense than that obtained with solution C.

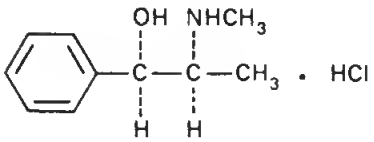
Assay. Dissolve about 0.2 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.68 mg of $C_{29}H_{40}N_2O_4,2HCl$.

EPHEDRINI HYDROCHLORIDUM
EPHEDRINE HYDROCHLORIDE

Molecular formula. $C_{10}H_{15}NO,HCl$

Relative molecular mass. 201.7

Graphic formula.



Chemical name. (–)-Ephedrine hydrochloride; [*R*-(*R**,*S**)]-α-[1-(methylamino)ethyl]benzenemethanol hydrochloride; CAS Reg. No. 50-98-6.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 4 parts of water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiasthmatic drug.

Storage. Ephedrine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Ephedrine hydrochloride darkens on exposure to light.

Requirements

Definition. Ephedrine hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{10}H_{15}NO,HCl$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.50 mg/ml solution, when observed between 230 nm and 350 nm, exhibits maxima at about 251 nm, 257 nm, and 263 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.37, 0.48, and 0.36, respectively.

B. Dissolve 10 mg in 1 ml of water and add 0.1 ml of copper(II) sulfate (80 g/l) TS, followed by 2 ml of sodium hydroxide (~80 g/l) TS; a violet colour is produced. Add 1 ml of ether R and shake; a purple colour is produced in the ethereal layer and a blue colour in the aqueous layer.

C. Dissolve 0.05 g in 5 ml of water. Add a few drops of sodium hydroxide (~80 g/l) TS and 4 ml of potassium ferricyanide (50 g/l) TS, and heat; an odour of benzaldehyde is perceptible.

D. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 217–220 °C.

Specific optical rotation. Use a 50 mg/ml solution; $[\alpha]_D^{20\,°C} = -33.0$ to $-35.5°$.

Sulfates. Dissolve 0.050 g in 40 ml of water and add 1.5 ml of hydrochloric acid (~70 g/l) TS and 1 ml of barium chloride (50 g/l) TS; no turbidity develops within 10 minutes.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear, or not more opalescent than opalescence standard TS2, and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity and alkalinity. Dissolve 1.0 g in 10 ml of water and add 0.1 ml of methyl red/ethanol TS; not more than 0.1 ml of sodium hydroxide (0.1 mol/l) VS or 0.1 ml of hydrochloric acid (0.1 mol/l) VS is required to obtain the mid-point of the indicator (orange).

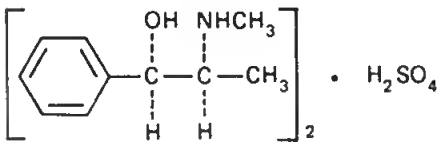
Assay. Dissolve about 0.2 g, accurately weighed, in 10 ml of warm mercuric acetate/acetic acid TS, add 50 ml of acetone R and 1 ml of methyl orange/acetone TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.17 mg of $C_{10}H_{15}NO,HCl$.

EPHEDRINI SULFAS
EPHEDRINE SULFATE

Molecular formula. $(C_{10}H_{15}NO)_2,H_2SO_4$

Relative molecular mass. 428.5

Graphic formula.



Chemical name. (–)-Ephedrine sulfate (2:1) (salt); [*R*-(*R**,*S**)]-α-[1-(methylamino)ethyl]benzenemethanol sulfate (2:1) (salt); CAS Reg. No. 134-72-5.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antiasthmatic drug.

Storage. Ephedrine sulfate should be kept in a well-closed container, protected from light.

Additional information. Ephedrine sulfate darkens on exposure to light. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ephedrine sulfate contains not less than 98.0% and not more than 101.0% of $(C_{10}H_{15}NO)_2,H_2SO_4$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 1.0 mg/ml solution, when observed between 230 nm and 350 nm, exhibits maxima at about 251 nm, 257 nm, and 262 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.61, 0.76, and 0.61, respectively.

B. Dissolve 10 mg in 1 ml of water and add 0.1 ml of copper(II) sulfate (80 g/l) TS, followed by 2 ml of sodium hydroxide (~80 g/l) TS; a violet colour is produced. Add 1 ml of ether R and shake; a purple colour is produced in the ethereal layer and a blue colour in the aqueous layer.

C. Dissolve 0.05 g in 5 ml of water. Add a few drops of sodium hydroxide (~80 g/l) TS and 4 ml of potassium ferricyanide (50 g/l) TS, and heat; an odour of benzaldehyde is perceptible.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

E. Melting temperature, about 245 °C with decomposition.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -30.5$ to -32.5 °.

Chlorides. A quantity of 0.20 g of the test substance shows no more turbidity than 0.40 ml of hydrochloric acid (0.02 mol/l) VS when subjected to the procedure described under 2.2.1 Limit test for chlorides; the chloride content is not more than 1.4 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear, or not more opalescent than opalescence standard TS2, and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Acidity and alkalinity. Dissolve 1.0 g in 10 ml of water and add 0.1 ml of methyl red/ethanol TS; not more than 0.1 ml of sodium hydroxide (0.1 mol/l) VS or 0.1 ml of hydrochloric acid (0.1 mol/l) VS is required to obtain the midpoint of the indicator (orange).

Assay. Dissolve about 0.3 g, accurately weighed, in 10 ml of water, add about 3 g of sodium chloride R to saturate the solution, then add 5 ml of sodium hydroxide (1 mol/l) VS and extract with 4 volumes, each of 25 ml, of chloroform R. Wash the combined chloroform extracts with 10 ml of a saturated solution of sodium chloride R, and filter through purified cotton saturated with chloroform R. Shake the aqueous wash solution with 10 ml of chloroform R and add it to the main chloroform extract. Add 0.25 ml of methyl red/ethanol TS and titrate with perchloric acid/dioxan (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid/dioxan (0.1 mol/l) VS is equivalent to 21.43 mg of $(C_{10}H_{15}NO)_2,H_2SO_4$.

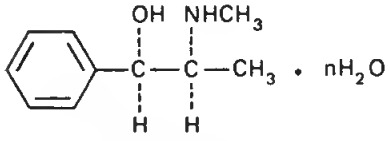
EPHEDRINUM
EPHEDRINE

Ephedrine, anhydrous
Ephedrine hemihydrate

Molecular formula. $C_{10}H_{15}NO$ (anhydrous); $C_{10}H_{15}NO, \frac{1}{2}H_2O$ (hemihydrate).

Relative molecular mass. 165.2 (anhydrous); 174.2 (hemihydrate).

Graphic formula.



n = 0 (anhydrous)
n = 1/2 (hemihydrate)

Chemical name. (–)-Ephedrine; [*R*-(*R**,*S**)]-α-[1-(methylamino)ethyl]benzenemethanol; CAS Reg. No. 299-42-3 (anhydrous).

(–)-Ephedrine hemihydrate; [*R*-(*R**,*S**)]-α-[1-(methylamino)ethyl]benzenemethanol hemihydrate; CAS Reg. No. 50906-05-3 (hemihydrate).

Description. Colourless crystals or a white, crystalline powder; odourless or with a slight, aromatic odour.

Solubility. Soluble in water; very soluble in ethanol (~750 g/l) TS; freely soluble in ether R.

Category. Antiasthmatic drug.

Storage. Ephedrine should be kept in a well-closed container, protected from light.

Labelling. The designation on the container should state whether the substance is the hemihydrate or is in the anhydrous form.

Additional information. Solutions of Ephedrine in chloroform R may become turbid, especially when the substance contains more than 10 mg of water per g. Even in the absence of light, Ephedrine is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Anhydrous Ephedrine melts at about 38 °C, whereas Ephedrine hemihydrate melts at about 42 °C.

Requirements

Definition. Ephedrine contains not less than 98.5% and not more than 101.0% of $C_{10}H_{15}NO$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 0.50 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits 3 maxima at about 251 nm, 257 nm, and 263 nm.

B. Dissolve 10 mg in 1 ml of water and add 0.1 ml of copper(II) sulfate (80 g/l) TS, followed by 2 ml of sodium hydroxide (~80 g/l) TS; a violet colour is produced. Add 1 ml of ether R and shake; a purple colour is produced in the ethereal layer and a blue colour in the aqueous layer.

C. Dissolve 0.05 g in 5 ml of water. Add a few drops of sodium hydroxide (~80 g/l) TS and 4 ml of potassium ferricyanide (50 g/l) TS, and heat; an odour of benzaldehyde is perceptible.

Specific optical rotation. Dissolve about 2.25 g, accurately weighed, in 15 ml of hydrochloric acid (~70 g/l) TS and dilute to 50 ml with water. Calculate the result with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = -41$ to -43 °.

Chlorides. Dissolve 0.70 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.35 mg/g.

Sulfates. Dissolve 1.2 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.4 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. For the anhydrous form use about 2 g of the substance; the water content is not more than 10 mg/g. For the hemihydrate use about 1 g of the substance; the water content is not less than 45 mg/g and not more than 55 mg/g.

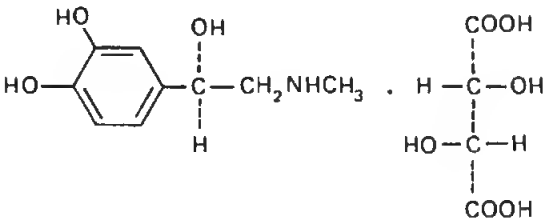
Assay. Dissolve about 0.5 g, accurately weighed, in 5 ml of ethanol (~750 g/l) TS, add 50.0 ml of hydrochloric acid (0.1 mol/l) VS and titrate with sodium hydroxide (0.1 mol/l) VS, using methyl red/ethanol TS as indicator. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 16.52 mg of $C_{10}H_{15}NO$.

EPINEPHRINI HYDROGENOTARTRAS
EPINEPHRINE HYDROGEN TARTRATE

Molecular formula. $C_9H_{13}NO_3,C_4H_6O_6$ or $C_{13}H_{19}NO_9$

Relative molecular mass. 333.3

Graphic formula.



Chemical name. (–)-(*R*)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol L-(+)-tartrate (1:1) (salt); (–)-(*R*)-4-[1-hydroxy-2-(methylamino)ethyl]-1,2-benzenediol[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (1:1) (salt); (–)-α-3,4-dihydroxyphenyl-β-(methylamino)ethanol L-(+)-tartrate; CAS Reg. No. 51-42-3.

Other name. Adrenaline tartrate. (In certain countries the name Adrenaline is a trademark. In those countries this name may be used only when applied to the product issued by the owners of the trademark.)

Description. A white to greyish white, crystalline powder; odourless.

Solubility. Soluble in 3 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Sympathomimetic.

Storage. Epinephrine hydrogen tartrate should be kept in a tightly closed container, protected from light.

Additional information. Epinephrine hydrogen tartrate gradually darkens in colour on exposure to air and light. Even in the absence of light, Epinephrine hydrogen tartrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Epinephrine hydrogen tartrate contains not less than 98.0% and not more than 101.0% of $C_9H_{13}NO_3,C_4H_6O_6$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.10 mg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 230 nm and 350 nm exhibits a maximum at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.8.

B. Dissolve 10 mg in 10 ml of water and transfer 1 ml to a flask containing 10 ml of buffer phthalate, pH 3.4, TS; another buffer having the same pH may also be used. Add 0.5 ml of iodine (0.1 mol/l) VS, and allow to stand for 5 minutes. Add 2 ml of sodium thiosulfate (0.1 mol/l) VS, and allow to stand for 1 minute; a strong red colour is produced (distinction from levarterenol, which gives a clear solution with a pink tinge).

C. The filtrate obtained when carrying out the determination of specific optical rotation (see below) yields reaction B described under 2.1 General identification tests, as characteristic of tartrates.

Specific optical rotation. Dissolve about 0.5 g in 20 ml of water containing about 0.1 g of sodium metabisulfite R, add a slight excess of ammonia (~100 g/l) TS, and allow to stand in the cold for 1 hour. Filter (keep the filtrate for identity test C, see above), wash the precipitate with 3 quantities, each of 2 ml of water, followed by 5 ml of ethanol (~750 g/l) TS and 5 ml of ether R. Dry the precipitate at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours. Use a 20 mg/ml solution of epinephrine base in hydrochloric acid (0.5 mol/l) VS; $[\alpha]_D^{20\,°C} = -50$ to $-53\,°$.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R for 3 hours; it loses not more than 5.0 mg/g.

Adrenalone. The absorbance of a 1-cm layer of a 4.0 mg/ml solution in hydrochloric acid (0.1 mol/l) VS at 310 nm is not more than 0.2 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Levarterenol. Dissolve 10 mg in 1 ml of water, add 4 ml of buffer borate, pH 9.6, TS, or another buffer having the same pH, mix, add 1 ml of a freshly prepared solution of sodium 1,2-naphthoquinone-4-sulfonate (5 g/l) TS, mix, and allow to stand for 30 minutes. Add 0.2 ml of benzalkonium chloride TS1, mix, add 15 ml of toluene R, previously washed with buffer borate, pH 9.6, TS and filtered through a dry filter-paper, shake for 30 minutes and allow to separate, centrifuging if necessary. Any red or purple colour in the toluene-layer is not more intense than that produced by treating a solution of 0.40 mg of levarterenol hydrogen tartrate R and 9.0 mg of epinephrine hydrogen tartrate R in 1 ml of water in a similar manner, when compared as described under 1.11 Colour of liquids.

Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of glacial acetic acid R1, warming slightly if necessary, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.33 mg of $C_9H_{13}NO_3,C_4H_6O_6$.

Additional requirements for Epinephrine hydrogen tartrate for parenteral use

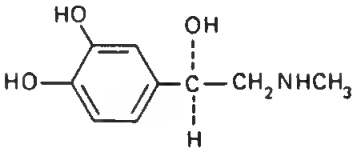
Complies with the monograph for "Parenteral preparations".

EPINEPHRINUM
EPINEPHRINE

Molecular formula. $C_9H_{13}NO_3$

Relative molecular mass. 183.2

Graphic formula.



Chemical name. (–)-(*R*)-3,4-Dihydroxy-α-[(methylamino)methyl]benzyl alcohol; (–)-(*R*)-4-[1-hydroxy-2-(methylamino)ethyl]-1,2-benzenediol; (–)-α-3,4-dihydroxyphenyl-β-methylaminoethanol; CAS Reg. No. 51-43-4.

Other name. Adrenaline. (In certain countries the name Adrenaline is a trademark. In those countries this name may be used only when applied to the product issued by the owners of the trademark.)

Description. A white or almost white, microcrystalline powder; odourless.

Solubility. Very slightly soluble in water; practically insoluble in ethanol (~750 g/l) TS, ether R and acetone R.

Category. Sympathomimetic.

Storage. Epinephrine should be kept in a hermetically closed container, protected from light.

Additional information. Epinephrine gradually darkens in colour on exposure to air and light. Even in the absence of light, Epinephrine is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Epinephrine contains not less than 98.5% and not more than 101.0% of $C_9H_{13}NO_3$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.030 mg/ml solution in hydrochloric acid (0.01 mol/l)VS, when observed between 230 nm and 350 nm exhibits a maximum at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.45 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

B. Dissolve 10 mg in 10 ml of hydrochloric acid (0.01 mol/l) VS and transfer 1 ml to a flask containing 10 ml of buffer phthalate, pH 3.4, TS; another buffer having the same pH may also be used. Add 0.5 ml of iodine (0.1 mol/l)

VS and allow to stand for 5 minutes. Add 2 ml of sodium thiosulfate (0.1 mol/l) VS, and allow to stand for 1 minute; a strong red colour is produced (distinction from levarterenol, which gives a clear solution with a pink tinge).

Specific optical rotation. Use a 40 mg/ml solution in hydrochloric acid (1 mol/l) VS; $[\alpha]_D^{20\,°C} = -50$ to $-53\,°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R for 18 hours; it loses not more than 10 mg/g.

pH value. pH of a 5.0 mg/ml solution in carbon-dioxide-free water R, above 7.5.

Adrenalone. The absorbance of a 1-cm layer of a 2.0 mg/ml solution in hydrochloric acid (0.1 mol/l) VS at 310 nm is not more than 0.2 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Levarterenol. Dissolve 5.0 mg in 1 ml of tartaric acid (5 g/l) TS, add 4 ml of buffer borate, pH 9.6, TS, or another buffer having the same pH, mix, add 1 ml of a freshly prepared solution of sodium 1,2-naphthoquinone-4-sulfonate (5 g/l) TS, mix, and allow to stand for 30 minutes. Add 0.2 ml of benzalkonium chloride TS1, mix, add 15 ml of toluene R, previously washed with buffer borate, pH 9.6, TS and filtered through a dry filter-paper, shake for 30 minutes and allow to separate, centrifuging if necessary. Treat a solution of 0.40 mg of levarterenol hydrogen tartrate R and 9.0 mg of epinephrine hydrogen tartrate R in 1 ml of water in a similar manner. Any red or purple colour in the toluene layer is not more intense than that of the reference solution when compared as described under 1.11 Colour of liquids.

Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 18.32 mg of $C_9H_{13}NO_3$.

Additional requirements for Epinephrine for parenteral use

Complies with the monograph for "Parenteral preparations".

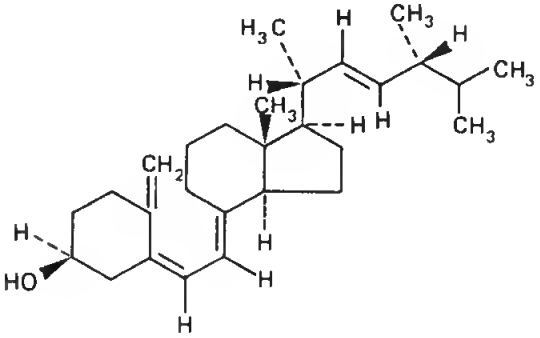
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 357.0 IU of endotoxin RS per mg.

ERGOCALCIFEROLUM
ERGOCALCIFEROL

Molecular formula. $C_{28}H_{44}O$

Relative molecular mass. 396.7

Graphic formula.



Chemical name. (5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraene-3β-ol; 24-methyl-9,10-secocholesta-5,7,10(19),22-tetraene-3β-ol; CAS Reg. No. 50-14-6.

Other name. Vitamin D_2.

Description. Colourless or slightly yellowish crystals or a white or slightly yellowish, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS, acetone R and ether R.

Category. Vitamin, antirachitic.

Storage. Ergocalciferol should be kept in a hermetically closed container, preferably in an inert atmosphere, such as nitrogen, protected from light and stored at a temperature between 2 and 8 °C.

Additional information. Ergocalciferol is affected by air and by light. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ergocalciferol contains not less than 95.0% and not more than 105.0% of $C_{28}H_{44}O$.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ergocalciferol RS or with the *reference spectrum* of ergocalciferol.

B. Dissolve about 2 mg in 10 ml of ethanol (~750 g/l) TS. To 1.0 ml add carefully 5 ml of sulfuric acid (~1760 g/l) TS and mix; a red colour is produced (distinction from colecalciferol, which gives a yellow colour).

C. Dissolve 5 mg in 5 ml of chloroform R, add about 0.5 ml of acetic anhydride R and about 0.1 ml of sulfuric acid (~1760 g/l) TS, shake well; the colour of the solution changes immediately from red to violet, then to blue, and finally to dark green.

D. Dissolve about 1 mg in 40 ml of dichloroethane R. To 1 ml of this solution add 4 ml of antimony trichloride TS; an orange colour is produced, which gradually becomes pink.

Melting range. 112–117 °C, determined without previous grinding or drying.

Specific optical rotation. Dissolve 0.2 g rapidly and without heating in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml. Determine the rotation within 30 minutes of preparation; $[\alpha]_D^{20\,°C} = +103$ to $+107$ °.

Ergosterol. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance, and as the mobile phase use a mixture of equal volumes of cyclohexane R and peroxide-free ether R, the mixture containing 0.10 mg of butylated hydroxytoluene R per ml. Apply separately to the plate 10 µl of the following solutions prepared immediately before use in a mixture of dichloroethane R containing 10 mg of squalane R and 0.10 mg of butylated hydroxytoluene R per ml: (A) 0.050 g of the test substance per ml, (B) 0.050 g of ergocalciferol RS per ml, (C) 0.10 mg of ergosterol R per ml; also apply to the plate 20 µl of solution (D) consisting of a mixture of equal volumes of solutions B and C. Develop the plate at once in the dark. After removing the plate from the chromatographic chamber, allow it to dry in air and spray it three times with antimony trichloride TS. Wait after spraying 3–4 minutes, then examine the chromatogram in daylight. The principal spot obtained with solution A is initially orange-yellow and then becomes brown; it corresponds in position, appearance and intensity with that obtained with solution B. Any violet spot obtained with solution A with an R_f value slightly less than that of the principal spot is not more intense than the spot obtained with solution C. The chromatogram obtained with solution A shows no additional spots compared with the chromatograms obtained with solutions B and C. The test is not valid unless the chromatogram obtained with solution D shows two clearly separated spots.

Reducing substances. Dissolve 0.10 g in 10 ml of aldehyde-free ethanol (~750 g/l) TS, add 0.5 ml of blue tetrazolium/ethanol TS and 0.5 ml of tetramethylammonium hydroxide/ethanol TS. Allow to stand for exactly 5 minutes and then add 1 ml of glacial acetic acid R. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. The absorbance is not greater than that obtained by repeating the operation with 10 ml of a solution containing 0.2 µg/ml of hydroquinone R in aldehyde-free ethanol (~750 g/l) TS.

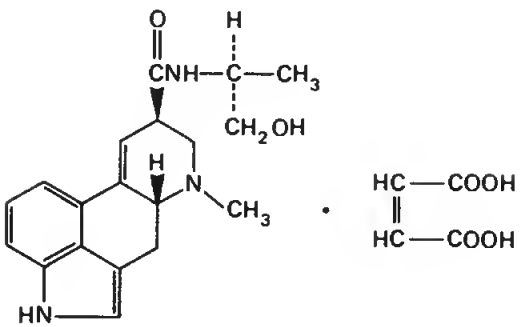
Assay. Dissolve rapidly and without heating 0.05 g, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 250 ml with the same solvent. Measure within 30 minutes of preparation the absorbance of a 1-cm layer of the diluted solution at the maximum at about 265 nm. Calculate the amount of $C_{28}H_{44}O$ in the substance being examined by comparison with ergocalciferol RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.48 ± 0.03.

ERGOMETRINI HYDROGENOMALEAS
ERGOMETRINE HYDROGEN MALEATE

Molecular formula. $C_{19}H_{23}N_3O_2,C_4H_4O_4$ or $C_{23}H_{27}N_3O_6$

Relative molecular mass. 441.5

Graphic formula.



Chemical name. 9,10-Didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8β-carboxamide maleate (1:1) (salt); 9,10-didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8β-carboxamide (*Z*)-2-butanedioate (1:1) salt; CAS Reg. No. 129-51-1.

Description. A white or faintly yellow, crystalline powder; odourless.

Solubility. Sparingly soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Oxytocic.

Storage. Ergometrine hydrogen maleate should be kept in a hermetically closed container, preferably in an inert atmosphere, such as nitrogen, protected from light and stored in a cool place.

Additional information. Ergometrine hydrogen maleate darkens in colour on exposure to light. Even in the absence of light, Ergometrine hydrogen maleate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ergometrine hydrogen maleate contains not less than 98.0% and not more than 101.0% of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ calculated with reference to the dried substance.

Identity tests

A. Dissolve 15 mg in 5 ml of water; a blue fluorescence is produced.

B. See the test described below under "Related alkaloids". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Dissolve 2 mg in 20 ml of water; to 1 ml of this solution add 2 ml of 4-dimethylaminobenzaldehyde TS1 and allow to stand for about 5 minutes; a deep blue colour is produced.

D. Dissolve 2 mg in 1 ml of water, and add 1 drop of bromine TS1; the colour of the reagent is discharged.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +50$ to $+56°$.

Clarity and colour of solution. A solution of 0.10 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour Yw3 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 20 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 3.0–5.0.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Place a sufficient volume of mobile phase to develop the chromatograms and a beaker containing 25 ml of ammonia (~260 g/l) TS into the chromatographic chamber, and equilibrate for 30 minutes. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 4.0 mg of the test substance per ml, and (B) 0.12 mg of the test substance per ml, and (C) 0.12 mg of ergometrine hydrogen maleate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with 4-dimethylaminobenzaldehyde TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.20 g, accurately weighed, in 20 ml of glacial acetic acid R1 and 10 ml of acetic anhydride R, and titrate with perchloric acid (0.05 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.05 mol/l) VS is equivalent to 22.07 mg of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

Additional requirements for Ergometrine hydrogen maleate for parenteral use

Complies with the monograph for "Parenteral preparations".

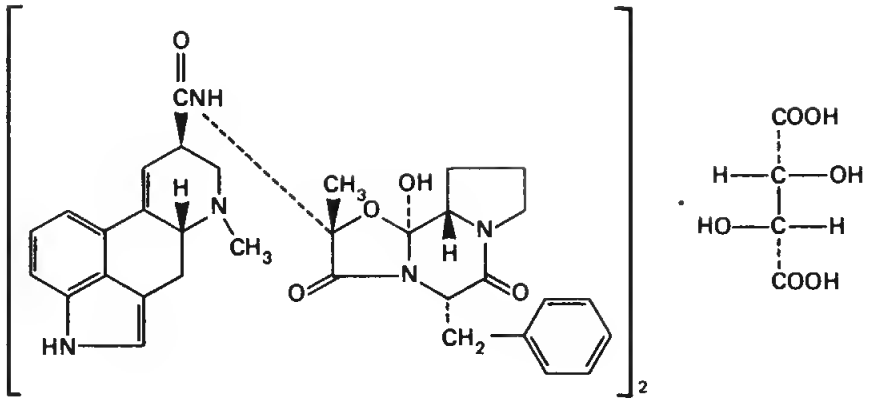
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 700.0 IU of endotoxin RS per mg.

ERGOTAMINI TARTRAS
ERGOTAMINE TARTRATE

Molecular formula. $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ or $C_{70}H_{76}N_{10}O_{16}$

Relative molecular mass. 1313

Graphic formula.



Chemical name. Ergotamine L-(+)-tartrate (2 : 1) (salt); 12′-hydroxy-2′-methyl-5′α-(phenylmethyl)ergotaman-3′,6′,18-trione[*R*-(*R**,*R**)]-2,3-dihydroxybutanedioate (2 : 1) (salt); CAS Reg. No. 379-79-3.

Description. Colourless crystals or a greyish white to yellowish white, crystalline powder; odourless.

Solubility. Slightly soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R, benzene R, and light petroleum R.

Category. Sympatholytic.

Storage. Ergotamine tartrate should be kept in a hermetically closed container, preferably in a inert atmosphere, such as nitrogen, protected from light and stored in a cool place.

Additional information. Even in the absence of light, Ergotamine tartrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ergotamine tartrate contains not less than 98.0% and not more than 101.0% of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$, calculated with reference to the dried substance.

Identity tests

A. See the test described below under "Related alkaloids". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

B. Dissolve 1 mg in a mixture of 5 ml of glacial acetic acid R and 5 ml of ethyl acetate R. To 1 ml of this solution add 1 ml of sulfuric acid (~1760 g/l) TS, with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of ferric chloride (25 g/l) TS, previously diluted with an equal volume of water; the red tinge becomes less apparent and the blue colour more pronounced.

Specific optical rotation of ergotamine base

- Prepare and use the solution rapidly, in subdued light.

Place about 0.35 g, accurately weighed, in a separator, dissolve it in 25 ml of tartaric acid (10 g/l) TS, add 0.5 g of sodium hydrogen carbonate R, and mix gently. Add 10 ml of ethanol-free chloroform R, shake vigorously, allow to separate, and filter the chloroform-layer through a small filter-paper previously moistened with ethanol-free chloroform R into a 50-ml volumetric flask. Repeat the extraction of the aqueous layer with three 10-ml portions of ethanol-free chloroform R, passing the extracts through the same filter. Place the flask in a water-bath at 20 °C for 10 minutes and adjust to 50 ml with the same solvent. Mix the solution and determine the optical rotation at 20 °C, preferably using tubes that can be controlled thermostatically. Separately, measure 25.0 ml of the solution, evaporate on a water-bath, and dry to constant weight at 95 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury). Calculate the specific optical rotation of the ergotamine base from the weight of the residue and the observed rotation; $[\alpha]_D^{20\,°C} = -150$ to -160 °.

Clarity and colour of solution. Add 25 mg of tartaric acid R to 50 mg of the test substance and dissolve at 20 °C in 10 ml of water; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Weigh the substance as rapidly as possible and dry to constant weight at 95 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 50 mg/g.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Place a sufficient volume of mobile phase to develop the chromatograms and a beaker containing 25 ml of ammonia (~260 g/l) TS into the chromatographic chamber, and equilibrate for 30 minutes. Apply separately to the plate 5 µl of each of 3 solutions in the mobile phase containing (A) 5.0 mg of the test substance per ml, (B) 0.25 mg of the test substance per ml, and (C) 0.25 mg of ergotamine tartrate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with 4-dimethylaminobenzaldehyde TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3g, accurately weighed, in 15ml of a mixture of 6 volumes of acetic anhydride R and 100 volumes of glacial acetic acid R1. Titrate with perchloric acid (0.05mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.05mol/l) VS is equivalent to 32.83mg of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

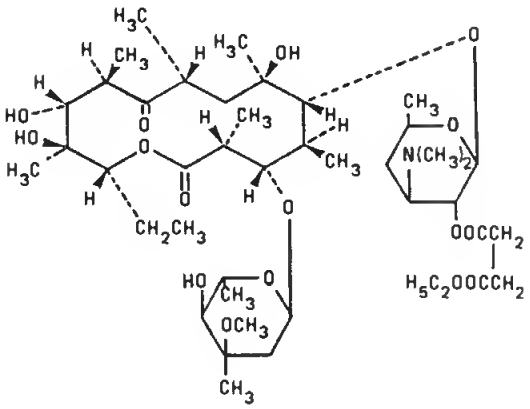
ERYTHROMYCINI ETHYLSUCCINAS
ERYTHROMYCIN ETHYLSUCCINATE

Erythromycin ethylsuccinate for parenteral use

Molecular formula. $C_{43}H_{75}NO_{16}$

Relative molecular mass. 862.1

Graphic formula.



Chemical name. Erythromycin 2′-(ethylsuccinate); erythromycin 2′(ethyl butanedioate); [3*R*-(3*R**,4*S**,5*S**,6*R**,7*R**,9*R**,11*R**,12*R**,13*S**,14*R**)]-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione 2′-(ethyl butanedioate); CAS Reg. No. 1264-62-6.

Description. A white or slightly yellow, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; soluble in ethanol (~750g/l) TS and macrogol 400R.

Labelling. The designation Erythromycin ethylsuccinate for parenteral use indicates that the substance complies with the additional requirement for Erythromycin ethylsuccinate and may be used for parenteral administration.

Category. Antibacterial drug.

Storage. Erythromycin ethylsuccinate should be kept in a tightly closed container, protected from light.

Requirements

Definition. Erythromycin ethylsuccinate contains not less than 740 International Units of erythromycin per mg, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from erythromycin ethylsuccinate RS or with the *reference spectrum* of erythromycin ethylsuccinate.

B. To 5 mg add 2 ml of sulfuric acid (~1760 g/l) TS and shake gently; a reddish brown colour is produced.

C. Dissolve 3 mg in 2 ml of acetone R and add 2 ml of hydrochloric acid (~420 g/l) TS; an orange colour is produced, which changes to orange-red and finally to purplish red. Add 2 ml of chloroform R and shake; the chloroform layer becomes blue.

D. To 5 mg add 5 ml of xanthydrol TS and heat on a water-bath; a red colour is produced.

Sulfated ash. Not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 30 mg/g.

pH value. Shake 0.5 g with 50 ml of carbon-dioxide-free water R; pH of the suspension, 6.0–8.5.

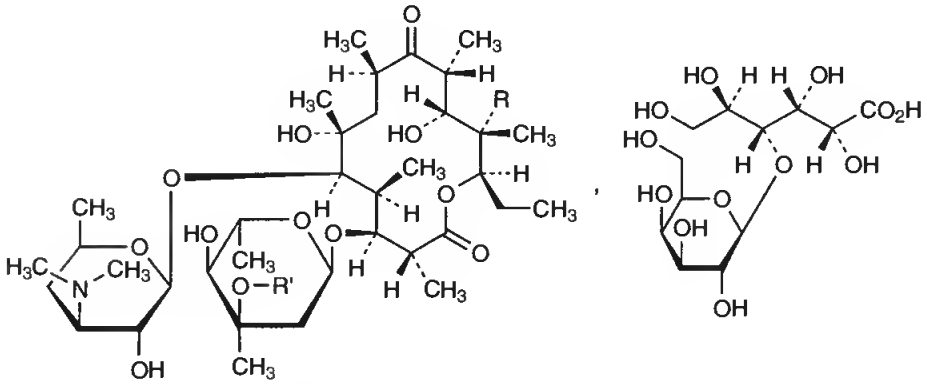
Assay. Dissolve 50 mg in sufficient methanol R to produce 100 ml and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using

Bacillus pumilus (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0 TS1 or TS2, an appropriate concentration of erythromycin (usually between 5 and 25 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 740 IU of erythromycin per mg, calculated with reference to the anhydrous substance.

Additional requirement for Erythromycin Ethylsuccinate for parenteral use

Complies with the monograph for "Parenteral preparations".

ERYTHROMYCINI LACTOBIONAS
ERYTHROMYCIN LACTOBIONATE



Erythromycin	R	R'
A	OH	CH_3
B	H	CH_3
C	OH	H

$C_{37}H_{67}NO_{13},C_{12}H_{22}O_{12}$

Relative molecular mass. 1092

Chemical name. Erythromycin lactobionate (1:1) (salt); erythromycin mono(4-*O*-β-D-galactopyranosyl-D-gluconate) (salt); CAS Reg. No. 3847-29-8.

Description. White or slightly yellow crystals or a white, crystalline powder; odour, faint.

Solubility. Freely soluble in water, ethanol (~750 g/l) TS and methanol R; slightly soluble in acetone R; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Erythromycin lactobionate should be kept in a tightly closed container, protected from light.

Additional information. Each mg of erythromycin lactobionate is equivalent to 0.6722 mg of erythromycin.

Requirements

Definition. Erythromycin lactobionate contains not less than 600 IU of erythromycin per mg, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from erythromycin lactobionate RS or with the *reference spectrum* of erythromycin lactobionate.

B. See the test described below under "Related substances". One of the two principal spots obtained with solution A corresponds in position with the principal spot obtained with solution B. The other principal spot corresponds in position with the principal spot obtained with solution D.

C. Dissolve 20 mg in 2.0 ml of water and cautiously add about 1 ml of sulfuric acid (~1760 g/l) TS to form a lower layer; a red-brown ring appears at the interface of the two liquids. Shake; a dark red-brown solution is produced.

D. Dissolve about 10 mg in 5 ml of hydrochloric acid (~250 g/l) TS; a yellowish green colour develops.

Clarity and colour of solution. A solution of 0.85 g in 10 ml of water is clear or not more opalescent than opalescence standard TS1, and colourless or not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of Erythromycin lactobionate; the water content is not more than 0.050 g/g.

Sulfated ash. Not more than 20 mg/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 6.0–7.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silanized silica gel R3 as the coating substance and a mixture of 5 volumes of methanol R and 3 volumes of ammonium acetate (50 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in methanol R containing (A) 3 mg of Erythromycin lactobionate per ml, (B) 2 mg of erythromycin RS per ml, (C) 0.10 mg of erythromycin RS per ml, and (D) 0.66 mg of lactobionic acid R per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray with anisaldehyde TS, heat at 110 °C for 5 minutes, and allow to cool. Examine the chromatogram in daylight.

Disregard the spot corresponding to lactobionic acid. Any spot obtained with solution A, other than the principal spot and any spot with a lower R_f-value, is not more intense than that obtained with solution B. Any spot obtained with solution A, other than the principal spot and any spot with a higher R_f-value, is not more intense than that obtained with solution C.

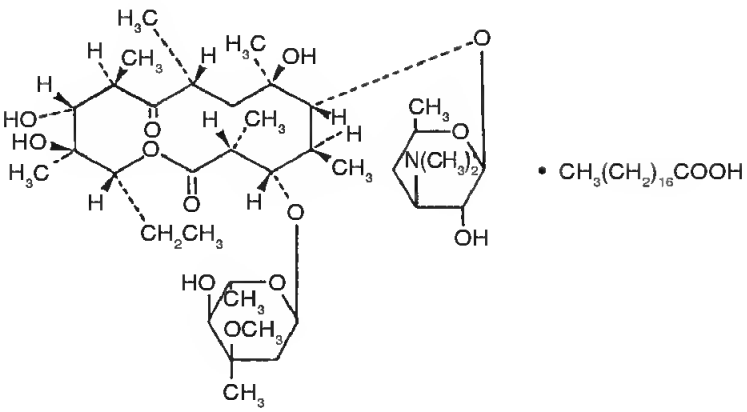
Assay. Carry out the determination as described under 3.1 Microbiological assay of antibiotics, using either (a) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS, an appropriate concentration of erythromycin (usually between 5 and 25 IU per ml), and an incubation temperature of 35–39 °C, or (b) *Micrococcus luteus* (ATCC 9341) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS1, or TS2, an appropriate concentration of erythromycin (usually between 0.5 and 1.5 IU per ml), and an incubation temperature of 32–35 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency.

ERYTHROMYCINI STEARAS
ERYTHROMYCIN STEARATE

Molecular formula. $C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$

Relative molecular mass. 1018

Graphic formula.



Chemical name. Erythromycin stearate (salt); erythromycin octadecanoate (salt); [3*R*-(3*R**,4*S**,5*S**,6*R**,7*R**,9*R**,11*R**,12*R**,13*S**,14*R**)]-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione octadecanoate (salt); CAS Reg. No. 643-22-1.

Description. Colourless or slightly yellow crystals or a white or slightly yellow powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS; soluble in methanol R, and ether R; these solutions may be opalescent.

Category. Antibacterial drug.

Storage. Erythromycin stearate should be kept in a tightly closed container, protected from light.

Requirements

Definition. Erythromycin stearate contains not less than 550 International Units of erythromycin per mg or not less than 77.0% of $C_{37}H_{67}NO_{13}$,$C_{18}H_{36}O_2$, both calculated with reference to the anhydrous substance.

Identity tests

- Either test A or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the

spectrum obtained from erythromycin stearate RS or with the *reference spectrum* of erythromycin stearate.

B. Dissolve 3 mg in 2 ml of acetone R and add 2 ml of hydrochloric acid (~420 g/l) TS; an orange colour is produced, which changes to red and then to deep purplish red. Add 2 ml of chloroform R and shake; the chloroform layer becomes purple.

C. To 5 mg add 5 ml of xanthydrol TS and heat on a water-bath; a red colour is produced.

D. Heat gently 0.1 g with 5 ml of hydrochloric acid (~70 g/l) TS and 10 ml of water until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer and heat it with 3 ml of sodium hydroxide (0.1 mol/l) VS; allow to cool; the solution sets to a gel. Add 10 ml of hot water and shake; the solution froths. To 1 ml add 1 ml of calcium chloride (~55 g/l) TS; a granular precipitate, insoluble in hydrochloric acid (~250 g/l) TS, is produced.

Erythromycin stearate. Shake 0.5 g with 30 ml of chloroform R. If the solution is clear, add 50 ml of glacial acetic acid R1, previously neutralized with perchloric acid (0.1 mol/l) VS, and continue with the titration as described below.

If the solution is opalescent, shake with an additional 2 quantities, each of 25 ml of chloroform R, filter each extract, wash the filter with chloroform R, and evaporate the combined filtrate and washings on a water-bath to about 30 ml. Add 50 ml of glacial acetic acid R1, previously neutralized with perchloric acid (0.1 mol/l) VS, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 101.8 mg of $C_{37}H_{67}NO_{13}$,$C_{18}H_{36}O_2$; the erythromycin stearate content is not less than 0.770 g/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.6 g of the substance; the water content is not more than 40 mg/g.

Free stearic acid. Dissolve 0.4 g in 50 ml of neutralized ethanol TS and titrate with sodium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically. Calculate the volume of sodium hydroxide (0.1 mol/l) VS required for each g of the substance and subtract the volume of perchloric acid (0.1 mol/l) VS required for each g of the substance in the test for erythromycin stearate. Each ml of the difference is equivalent to 28.45 mg of $C_{18}H_{36}O_2$; not more than 185 mg/g.

Sodium stearate. Moisten 2.0 g in a platinum dish with a small quantity of sulfuric acid (~1760 g/l) TS, ignite gently, again moisten with sulfuric acid (~1760 g/l) TS, ignite at about 800 °C, cool, and weigh. Each g of residue is equivalent to 4.317 g of $C_{18}H_{35}NaO_2$; not more than 60 mg/g.

Total stearic acid, stearates and water. Using the results of the above four determinations, add together the percentages of free stearic acid, erythromycin stearate, sodium stearate (all calculated with reference to the undried substance) and the water content; the total is not less than 98.0% and not more than 103.0%.

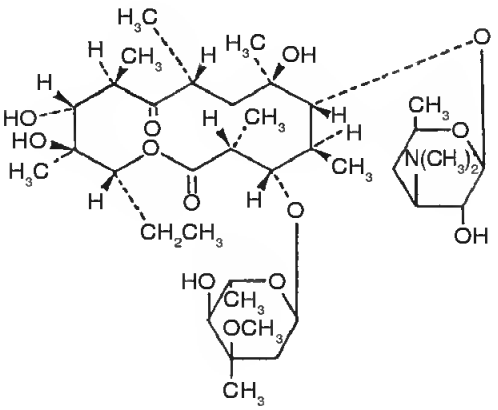
Assay. Dissolve 50 mg in sufficient methanol R to produce 100 ml and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0 TS1 or TS2, an appropriate concentration of erythromycin (usually between 5 and 25 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 550 IU of erythromycin per mg, calculated with reference to the anhydrous substance.

ERYTHROMYCINUM
ERYTHROMYCIN

Molecular formula. $C_{37}H_{67}NO_{13}$

Relative molecular mass. 733.9

Graphic formula.



Chemical name. [3*R*-(3*R**,4*S**,5*S**,6*R**,7*R**,9*R**,11*R**,12*R**,13*S**,14*R**)]-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxyl]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethy-

lamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione; CAS Reg. No. 114-07-8.

Description. White or slightly yellow crystals or powder; odourless or almost odourless.

Solubility. Soluble in 1000 parts of water but less soluble in hot water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Antibacterial drug.

Storage. Erythromycin should be kept in a tightly closed container, protected from light.

Additional information. Erythromycin is slightly hygroscopic.

Requirements

Definition. Erythromycin is a mixture of substances produced by the growth of certain strains of *Streptomyces erythreus*. The main component of the mixture is erythromycin A with lesser amounts of erythromycins B and C.

- The molecular formula, the relative molecular mass, and the chemical name given above relate to erythromycin A only.

Erythromycin contains not less than 870 International Units per mg, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption is concordant with the spectrum obtained from erythromycin RS or with the *reference spectrum* of erythromycin.

B. To 5 mg add 2 ml of sulfuric acid (~1760 g/l) TS and shake gently; a reddish brown colour is produced.

C. Dissolve 3 mg in 2 ml of acetone R and add 2 ml of hydrochloric acid (~420 g/l) TS; an orange colour is produced, which changes to red and then to deep purplish red. Add 2 ml of chloroform R and shake; the chloroform layer becomes purple.

D. To 5 mg add 5 ml of xanthydrol TS and heat on a water-bath; a red colour is produced.

Specific optical rotation. Use a 20 mg/ml solution in dehydrated ethanol R, allow to stand for 30 minutes, measure the angle of rotation, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = -71$ to $-78\,°$.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 1 g of the substance; the water content is not more than 100 mg/g.

pH value. Dissolve 0.1 g in 50 ml of a mixture composed of 1 volume of methanol R and 19 volumes of carbon-dioxide-free water R; the pH is between 8.0 and 10.5.

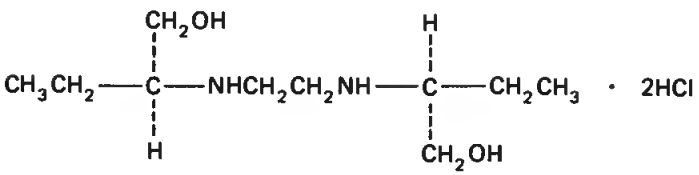
Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0 TS or TS2, an appropriate concentration of erythromycin (usually between 5 and 25 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 870 IU per mg, calculated with reference to the anhydrous substance.

ETHAMBUTOLI HYDROCHLORIDUM
ETHAMBUTOL HYDROCHLORIDE

Molecular formula. $C_{10}H_{24}N_2O_2,2HCl$

Relative molecular mass. 277.2

Graphic formula.



Chemical name. (+)-(*S,S*)-2,2′-(Ethylenediimino)di-1-butanol dihydrochloride; [*S*-(*R**,*R**)]-2,2′-(1,2-ethanediyldiimino)bis[1-butanol] dihydrochloride; CAS Reg. No. 1070-11-7.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 1 part of water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antibacterial (tuberculostatic).

Storage. Ethambutol hydrochloride should be kept in a well-closed container.

Requirements

Definition. Ethambutol hydrochloride contains not less than 98.0% and not more than 100.5% of $C_{10}H_{24}N_2O_2,2HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ethambutol hydrochloride RS or with the *reference spectrum* of ethambutol hydrochloride.

B. See the test described below under "2(*R*-Aminobutanol)". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. A 0.1 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

D. Melting temperature, about 200 °C.

Specific optical rotation. Use a 0.10 g/ml solution; $[\alpha]_D^{20\,°C}$ = +5.0 to 7.0 °.

Heavy metals. Use 1.0 g for the preparation Of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.01 g/ml solution, 3.0–4.5.

2(*R*-Aminobutanol). Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 11 volumes of ethyl acetate R, 7 volumes of glacial acetic acid R, 1 volume of hydrochloric acid (~420 g/l) TS, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of 3 solutions in methanol R containing (A) 50 mg of the test substance per ml, (B) 0.50 mg of the test substance per ml, and (C) 0.50 mg of 2-aminobutanol R per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, heat at 105 °C for 5 minutes, cool, spray with triketohydrindene/cadmium TS, heat at 90 °C for 5 minutes, and examine the chromatogram in daylight. The spot obtained with solution C is more intense than any spot, corresponding in position and appearance, obtained with solution A.

Assay. Dissolve about 0.3 g, accurately weighed, in 100 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 13.86 mg of $C_{10}H_{24}N_2O_2,2HCl$.

ETHANOLUM
ETHANOL

$H_3C—CH_2—OH$

C_2H_6O

Relative molecular mass. 46.07

Chemical name. Ethyl alcohol; ethanol; CAS Reg. No. 64-17-5.

Other name. Absolute alcohol, dehydrated alcohol.

Description. A colourless, clear and mobile liquid; odour, characteristic.

Miscibility. Miscible with water and ether R.

Category. Solvent; antiseptic.

Storage. Ethanol should be kept in a well-closed container, and stored whenever possible at a temperature between 8 and 15 °C.

Additional information. Ethanol is flammable, burning with a blue smokeless flame. Hygroscopic. Boiling point, about 79 °C.

Requirements

Ethanol contains not less than **98.8% *v/v*** and not more than the equivalent of **100.0% *v/v*** of C_2H_6O, corresponding to not less than **98.1% *m/m*** and not more than the equivalent of **100.0% *m/m*** of C_2H_6O.

Identity tests

A. Place 0.25 ml in a small beaker, add 1 ml of potassium permanganate (10 g/l) TS and 0.5 ml of sulfuric acid (0.5 mol/l) VS, and cover the beaker immediately with a filter-paper moistened with a recently prepared solution of 0.1 g of sodium nitroprusside R and 0.5 g of piperazine hydrate R in 5 ml of water; a dark blue colour is produced on the filter-paper, that fades after a few minutes.

B. To a few drops add 1 ml of sulfuric acid (~1760 g/l) TS and a few drops of potassium dichromate (100 g/l) TS; a green colour is produced and an odour of acetaldehyde is perceptible.

Relative density. $d_{20}^{20} = 0.7904 - 0.7935$.

Non-volatile residue. Place 100 ml in a porcelain dish and heat on a water-bath until volatilized, dry the residue at 105 °C for 1 hour, and weigh; not more than 5 mg.

Water-insoluble substances. Dilute a volume of Ethanol with an equal volume of water; the mixture is clear and, after cooling to 10 °C, it remains clear for 30 minutes.

Acidity. Add 20 ml of carbon-dioxide-free water R and 3 drops of phenolphthalein/ethanol TS to 20 ml of Ethanol; the colour remains unchanged. Titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS; not more than 0.5 ml is required to obtain the midpoint of the indicator (pink).

Aldehydes and other foreign organic substances. Thoroughly clean a glass-stoppered cylinder with hydrochloric acid (~250 g/l) TS, rinse with water and the Ethanol to be examined. Place 20 ml of Ethanol in the cylinder. Cool the contents to about 15 °C and, by means of a carefully cleaned pipette, add 0.1 ml of potassium permanganate (0.02 mol/l) VS, noting the time of the addition. Mix at once by inverting the stoppered cylinder, and allow to stand at 15 °C for exactly 5 minutes; the pink colour does not entirely disappear.

Fusel oil and allied impurities. Allow 25 ml to evaporate spontaneously from a porcelain dish, carefully protected from dust, until the surface of the dish is barely moist; no foreign odour is perceptible, and on the addition of a few drops of sulfuric acid (~1760 g/l) TS, no red or brown colour develops.

Methanol. To 1 drop add 1 drop of water, 1 drop of phosphoric acid (~105 g/l) TS, and 2 drops of potassium permanganate (25 g/l) TS. Mix, allow to stand for 1 minute, and add, drop by drop, sodium metabisulfite (50 g/l) TS until the permanganate colour is discharged. If a brown colour remains, add 1 drop of phosphoric acid (~105 g/l) TS. To the colourless solution add 5 ml of freshly prepared disodium chromotropate TS, and heat on a water-bath at 60 °C for 10 minutes; no violet colour appears.

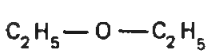
Benzene. Record an absorption spectrum of the Ethanol in a 1-cm layer against water between 220 nm and 350 nm. The absorbance at about 220 nm is not more than 0.30, at about 230 nm not more than 0.18, at about 240 nm not more than 0.08, and at about 270 to 350 nm not more than 0.02. A curve drawn through these points is smooth.

ETHER ANAESTHESICUS
ANAESTHETIC ETHER

Molecular formula. $C_4H_{10}O$

Relative molecular mass. 74.12

Graphic formula.



Chemical name. Ethyl ether; 1,1′-oxybis[ethane]; diethyl ether; CAS Reg. No. 60-29-7.

Description. A clear, colourless, volatile, very mobile liquid; odour, characteristic.

Miscibility. Miscible with 10 parts of water; miscible with ethanol (~750 g/l) TS.

Category. General anaesthetic.

Storage. Anaesthetic Ether should be kept in a securely closed, dry container, protected from light, at a temperature not exceeding 15 °C, and in quantities of not more than 1 kg.

Labelling. The designation on the container of anaesthetic Ether must state: "Highly flammable. Do not use near an open flame or other sources of heat that may cause ignition." The name and quantity of any antioxidant added must be stated.

Additional information. Anaesthetic Ether may contain a suitable antioxidant. It must not be used for anaesthesia if it has been removed from its original container for longer than 24 hours. Ether remaining in partially used containers may deteriorate rapidly. CAUTION: Vapours of ether mixed with air, oxygen, or nitrous oxide in certain concentrations are explosive. Do not use an open flame at any time during the testing procedure.

Requirements

Distillation range

- It is dangerous to determine the distillation range of the test liquid if it does not comply with the test for Peroxides.

Use a suitable heating device, and take precautions to avoid superheating the distillation flask above the level of the test liquid; it distils completely between 34.0 and 35.0 °C.

Relative density. $d_{20}^{20} = 0.713 - 0.716$.

Non-volatile residue

- It is dangerous to determine the non-volatile residue of the test liquid if it does not comply with the test for Peroxides.

Allow 50 ml to evaporate, dry the residue at 105 °C for 1 hour and weigh; it leaves not more than 20 µg/ml.

Acidity. Place 10 ml of ethanol (~750 g/l) TS in a 50-ml glass-stoppered flask, add 0.5 ml of phenolphthalein/ethanol TS and just sufficient carbonate-free sodium hydroxide (0.02 mol/l) VS to produce a pink colour that persists after shaking the mixture for 30 seconds; add 25 ml of the test liquid, mix gently, and add carbonate-free sodium hydroxide (0.02 mol/l) VS until the pink colour persists after shaking the mixture for 30 seconds; not more than 0.4 ml of additional carbonate-free sodium hydroxide is required.

Peroxides. To 10 ml of the test liquid add 2.0 ml of vanadium/sulfuric acid TS and shake. Separately prepare a reference solution by diluting 1.0 ml of hydrogen peroxide (~60 g/l) TS to 100 ml with water. To 0.10 ml of this reference solution add 2.0 ml of vanadium/sulfuric acid TS. The colour produced in the aqueous layer in the tube containing the test liquid, when viewed transversely against a white background, is not more intense than that of the reference solution when compared as described under 1.11 Colour of liquids.

Acetone and aldehydes. Transfer 2 ml of alkaline potassio-mercuric iodide TS to a glass-stoppered test-tube of about 12 ml capacity and about 1.5 cm diameter, and add 10 ml of the test liquid. Shake the tube vigorously for 10

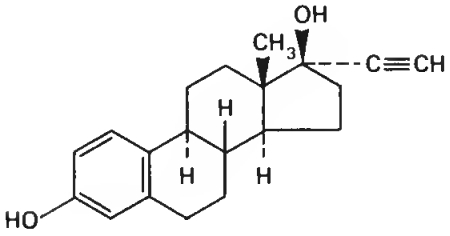
seconds and allow to stand in the dark for 5 minutes; no turbidity is produced. If the test liquid does not comply with this requirement, distil 40 ml, previously ensuring that it complies with the test for Peroxides, until only 5 ml remains; repeat the test on the distillate.

ETHINYLESTRADIOLUM
ETHINYLESTRADIOL

Molecular formula. $C_{20}H_{24}O_2$

Relative molecular mass. 296.4

Graphic formula.



Chemical name. 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol; 17-ethynyl-estra-1,3,5,(10)-triene-3,17β-diol; CAS Reg. No. 57-63-6.

Description. A white to slightly yellowish white, crystalline powder; odourless.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS; soluble in acetone R, and dioxan R.

Category. Estrogen.

Storage. Ethinylestradiol should be kept in a well-closed container, protected from light.

Additional information. Ethinylestradiol may exist in 2 polymorphic forms one of which melts at about 183 °C, the other, metastable, at about 143 °C.

Requirements

Definition. Ethinylestradiol contains not less than 97.0% and not more than 102.0% of $C_{20}H_{24}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ethinylestradiol RS or with the *reference spectrum* of ethinylestradiol. If the spectrum obtained from the solid state of the test substance is not concordant with the spectrum obtained from the reference substance, compare the spectra of solutions in chloroform R containing 30 mg/ml, using a path length of 0.2 mm.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use toluene R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 1.0 mg of the test substance per ml, and (B) 1.0 mg of ethinylestradiol RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with 4-toluenesulfonic acid/ethanol TS, and then heat at 120 °C for 5–10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 4.0 mg/ml solution in pyridine R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -27.0$ to $-30.0°$.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Estrone. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 92 volumes of dichloroethane R, 8 volumes of methanol R, and 0.5 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 freshly prepared solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 20 mg of the test substance per ml, and (B) 0.20 mg of estrone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the odour of the solvent is no longer detectable; then heat at 110 °C for 10 minutes. Spray the hot plate with sulfu-

ric acid/ethanol TS, heat again at 110 °C for 10 minutes, and examine the chromatogram in ultraviolet light (365 nm). The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

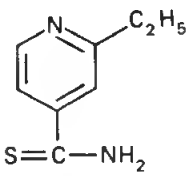
Assay. Dissolve about 0.05 g, accurately weighed, in sufficient dehydrated ethanol R to produce 100 ml, and dilute 10.0 ml of this solution to 50.0 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 281 nm. Calculate the amount of $C_{20}H_{24}O_2$ in the substance being tested by comparison with ethinylestradiol RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.72 ± 0.04.

ETHIONAMIDUM
ETHIONAMIDE

Molecular formula. $C_8H_{10}N_2S$

Relative molecular mass. 166.2

Graphic formula.



Chemical name. 2-Ethylthioisonicotinamide; 2-ethyl-4-pyridinecarbothioamide; CAS Reg. No. 536-33-4.

Description. Small yellow crystals or a yellow, crystalline powder; odour, slight.

Solubility. Practically insoluble in water; soluble in methanol R; sparingly soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Antileprosy drug.

Storage. Ethionamide should be kept in a tightly closed container, protected from light, and stored in a cool place.

Additional information. Ethionamide darkens on exposure to light.

Requirements

Definition. Ethionamide contains not less than 98.0% and not more than 101.0% of $C_8H_{10}N_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ethionamide RS or with the *reference spectrum* of ethionamide.

B. Mix 0.05 g with 0.10 g of 2,4-dinitrochlorobenzene R, then transfer 10 mg of this mixture to a test-tube and heat until melted. Cool and add 3 ml of potassium hydroxide/ethanol TS1; a red to orange-red colour is produced.

C. Heat 0.1 g with 5 ml of hydrochloric acid (1 mol/l) VS; the vapours evolved blacken lead acetate paper R.

D. Melting temperature, about 162 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in acetone R containing (A) 20 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.04 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Not more than one of any such spots is more intense than that obtained with solution C.

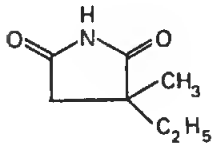
Assay. Dissolve about 0.15 g, accurately weighed, in 50 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 16.62 mg of $C_8H_{10}N_2S$.

ETHOSUXIMIDUM
ETHOSUXIMIDE

Molecular formula. $C_7H_{11}NO_2$

Relative molecular mass. 141.2

Graphic formula.



Chemical name. 2-Ethyl-2-methylsuccinimide; 3-ethyl-3-methyl-2,5-pyrrolidinedione; CAS Reg. No. 77-67-8.

Description. A white or almost white powder or waxy solid; odourless or with a faint characteristic odour.

Solubility. Freely soluble in water; very soluble in ethanol (~750 g/l) TS and ether R.

Category. Anticonvulsant.

Storage. Ethosuximide should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Ethosuximide is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ethosuximide contains not less than 99.0% and not more than 100.5% of $C_7H_{11}NO_2$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ethosuximide RS or with the *reference spectrum* of ethosuximide. If the spectrum obtained from the solid state of the test sub-

stance is not concordant with the spectrum obtained from the reference substance, dissolve a small amount of the substance in ethanol (~750 g/l) TS, evaporate to dryness on a water-bath, and prepare a potassium bromide disc as described in Method 3. Then compare the spectrum obtained from the disc with that of the reference substance.

B. Heat 0.1 g with 0.2 g of resorcinol R and 2 drops of sulfuric acid (~1760 g/l) TS at 140 °C for 5 minutes, allow to cool, add 5 ml of water, make alkaline with sodium hydroxide (~80 g/l) TS, and pour a few drops into a large volume of water; a bright green fluorescence is obtained.

C. Melting temperature, about 46 °C.

Cyanides. Dissolve 1 g in 10 ml of ethanol (~750 g/l) TS, add 3 drops of ferrous sulfate (15 g/l) TS, 1 ml of sodium hydroxide (~80 g/l) TS and a few drops of ferric chloride (25 g/l) TS. Warm gently, and acidify with sulfuric acid (~100 g/l) TS; no blue precipitate or blue colour is produced within 15 minutes.

Sulfated ash. Not more than 5.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 5.0 mg/g.

Acidity. Dissolve 5.0 g in 50 ml of water by warming on a water-bath for 5 minutes. Cool and titrate with sodium hydroxide (0.1 mol/l) VS, using bromocresol green/ethanol TS as indicator; not more than 0.7 ml is required to obtain the midpoint of the indicator (green).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of acetone R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per ml and (B) 0.050 mg of the test substance per ml. After removing the plate from the chromatographic chamber allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.28 g, accurately weighed, in 30 ml of dimethylformamide R, add 3 drops of azo violet TS and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS to a blue endpoint, as described under 2.6 Non-aqueous titration. Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 14.12 mg of $C_7H_{11}NO_2$.

ETHYLCELLULOSUM
ETHYLCELLULOSE

Chemical name. Cellulose ethyl ether; CAS Reg. No. 9004-57-3.

Description. A white to light tan, free-flowing powder.

Solubility. Practically insoluble in water, glycerol R, and propylene glycol R. When containing less than 46.5% of ethoxy groups, it is freely soluble in tetrahydrofuran R. When containing not less than 46.5% of ethoxy groups, it is freely soluble in ethanol (~750 g/l) TS, methanol R, toluene R, and ethyl acetate R.

Category. Film-coating agent; tablet binder; release-rate modifier for oral formulations.

Storage. Ethylcellulose should be kept in a well-closed container.

Labelling. The designation on the container of Ethylcellulose should state its viscosity.

Requirements

Definition. Ethylcellulose is an ethyl ether of cellulose.

Ethylcellulose contains not less than **44.0%** and not more than the equivalent of **51.0%** of ethoxy ($—OC_2H_5$) groups.

Identity tests

A. Dissolve 5 g in 95 g of a mixture by mass of 80 parts of toluene R and 20 parts of ethanol (~750 g/l) TS; a slightly yellow, clear, stable solution is produced. (Keep the solution for test B.)

B. Pour a few ml of the above solution onto a glass plate and allow the solvent to evaporate; a thin, though continuous, clear film is formed. Remove the film from the plate; it is flammable.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 40 µg/g.

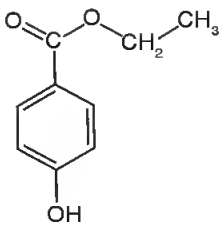
Sulfated ash. Not more than 4.0 mg/g.

Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 30 mg/g.

Assay. Carry out the assay as described under 2.9 Determination of methoxyl, using about 0.05 g, previously dried and accurately weighed.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 0.7510 mg of ($-OC_2H_5$).

ETHYLIS HYDROXYBENZOAS
ETHYL HYDROXYBENZOATE



$C_9H_{10}O_3$

Relative molecular mass. 166.2

Chemical name. Ethyl *p*-hydroxybenzoate; ethyl 4-hydroxybenzoate; CAS Reg. No. 120-47-8.

Other name. Ethylparaben.

Description. Small colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; sparingly soluble in boiling water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Antimicrobial preservative.

Storage. Ethyl hydroxybenzoate should be kept in a well-closed container.

Additional information. Ethyl hydroxybenzoate is normally used in combination with other hydroxybenzoates.

Requirements

Ethyl hydroxybenzoate contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_9H_{10}O_3$ calculated with reference to the dried substance.

Identity tests

A. Complies with the test under "Melting range".

B. To 0.5 g add 5 ml of sodium hydroxide (~80 g/l) TS, and heat in a water-bath for 5 minutes. After cooling, add 6 ml of sulfuric acid (~190 g/l) TS, collect the precipitate on a filter, wash thoroughly with a small amount of water, and dry over silica gel, desiccant, R. Melting temperature, about 214 °C.

Melting range. 115–118 °C.

Sulfated ash. Not more than 1.0 mg/g.

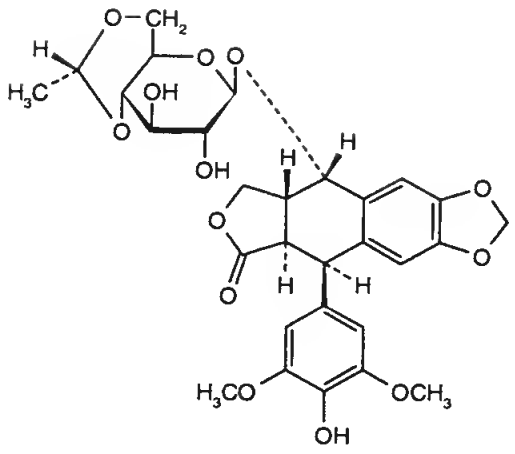
Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 2 hours; it loses not more than 5.0 mg/g.

Acidity. Dissolve 0.2 g in 5 ml of ethanol (~750 g/l) TS, add 5 ml of carbon-dioxide-free water R, and titrate with sodium hydroxide (0.1 mol/l) VS, using 0.1 ml of bromocresol green/ethanol TS as indicator; not more than 0.1 ml is required to obtain the midpoint of the indicator (green).

Assay. Place about 0.08 g, accurately weighed, in a ground-glass-stoppered flask, add 25 ml of sodium hydroxide (~80 g/l) TS, and boil gently under a reflux condenser for 30 minutes. Allow to cool, add 25 ml of potassium bromate (0.0333 mol/l) VS, 5 ml of potassium bromide (125 g/l) TS, and 40 ml of glacial acetic acid R. Cool in ice-water and add 10 ml of hydrochloric acid (~420 g/l) TS. Stopper the flask immediately and allow to stand for 15 minutes. Add 30 ml of potassium iodide (80 g/l) TS, close the flask, and mix. Titrate with sodium thiosulfate (0.1 mol/l) VS, using 2 ml of starch TS as indicator, added towards the end of the titration. Repeat the procedure without the Ethyl hydroxybenzoate being examined and make any necessary corrections.

One volume of sodium thiosulfate (0.1 mol/l) VS corresponds to two volumes of potassium bromate (0.0333 mol/l) VS. Each ml of potassium bromate (0.0333 mol/l) VS is equivalent to 5.540 mg of $C_9H_{10}O_3$.

ETOPOSIDUM
ETOPOSIDE



$C_{29}H_{32}O_{13}$

Relative molecular mass. 588.6

Chemical name. 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-Dglucopyranoside); [5*R*-[5α,5aβ,8aα,9β(*R**)]]-9-[(4,6-*O*-ethylidene-b-β-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethyoxyphenyl) furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one; CAS Reg. No. 33419-42-0.

Description. A white or almost white, crystalline powder.

Solubility. Practically insoluble in water; sparingly soluble in methanol R; slightly soluble in ethanol (~750 g/l) TS and dichloromethane R.

Category. Cytotoxic drug.

Storage. Etoposide should be kept in a tightly closed container.

Labelling. The designation Etoposide for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. *CAUTION*: Etoposide must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Etoposide contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{29}H_{32}O_{13}$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from etoposide RS or with the *reference spectrum* of etoposide.

B. See the test described below under "Related substances". The principal band obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Transfer about 5 mg to a test-tube and dissolve in 5 ml of glacial acetic acid R, add about 0.1 ml of ferric chloride (50 g/l) TS, and mix. Cautiously add about 2 ml of sulfuric acid (~1760 g/l) TS. Without mixing allow to stand for about 30 minutes; a pink to reddish brown ring develops at the interface and the upper layer is yellow.

D. Dissolve 5 mg in 5 ml of methanol R, add 5 ml of hydrochloric acid (~70 g/l) TS, and evaporate to dryness on a water-bath. To the residue add 20 ml of water and 10 ml of dichloromethane R, and shake vigorously. Allow to separate and to 1.0 ml of the aqueous layer add 2.0 ml of anthrone TS2 and mix; a blue-green colour is produced.

Specific optical rotation. Dissolve 0.050 g in 10 ml of a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R; $[\alpha]_D^{20\,°C} = -106°$ to $-114°$, calculated with reference to the dried substance.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Solution in methanol/dichloromethane. Dissolve 0.6 g in 20 ml of a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R; the solution is clear and not more intensely coloured than standard colour solution 2 of the most appropriate hue, when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 30 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 100 volumes of dichloromethane R, 20 volumes of acetone R, 8 volumes of

glacial acetic acid R, and 1.5 volumes of water as the mobile phase. Apply separately to the plate 5 µl, spread to form 10-mm bands, of each of 5 solutions in a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R containing (A) 0.050 g of Etoposide per ml, (B) 5 mg of Etoposide per ml, (C) 5 mg of etoposide RS per ml, (D) 0.25 mg of etoposide RS per ml, and (E) 0.10 mg of etoposide RS per ml. After removing the plate from the chromatographic chamber, dry it in a current of warm air for 5 minutes. Spray with a mixture of 1 volume of sulfuric acid (~1760 g/l) TS and 9 volumes of ethanol (~750 g/l) TS, and heat at 140 °C for 15 minutes. Cover the plate immediately with a glass plate of the same size. Examine the chromatogram in daylight.

Any band obtained with solution A, other than the principal band, is not more intense than that obtained with solution D. Furthermore, not more than two such bands are more intense than the band obtained with solution E (0.2%).

Assay. Determine as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (10 µm). Prepare a diluted solution of acetic acid as follows to be used in the mobile phase and for the preparation of solution C: to 96 ml of water add 4 ml of glacial acetic acid R. As the mobile phase, use a mixture of 76 volumes of the diluted acetic acid solution and 24 volumes of acetonitrile R.

Prepare the following solutions in methanol R: solution (A) 1.0 mg of Etoposide per ml; solution (B) 1.0 mg of etoposide RS per ml; and for solution (C) add 0.1 ml of the diluted acetic acid solution described above and 0.1 ml of phenolphthalein/ethanol TS to 10 ml of solution A, then add sodium hydroxide (1 mol/l) VS until the solution becomes faintly pink (about 0.15 ml), allow to stand for 15 minutes, and add 0.1 ml of the diluted acetic acid solution described above.

Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 285 nm.

Inject 10 µl of solution C. Allow the chromatography to continue until the peak corresponding to phenolphthalein is eluted. Phenolphthalein has a retention time relative to etoposide of about 2.7. Disregard any peak due to phenolphthalein. The assay is not valid unless the chromatogram shows two principal peaks and the resolution between these peaks is at least 1.5. If necessary, reduce the concentration of acetonitrile R in the mobile phase or reduce the flow rate to achieve the required resolution.

Inject alternately 10 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{29}H_{32}O_{13}$.

Additional requirements for Etoposide for parenteral use

Complies with the monograph for "Parenteral preparations".

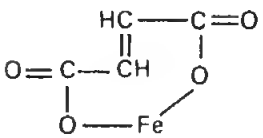
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.0 IU of endotoxin RS per mg.

FERROSI FUMARAS
FERROUS FUMARATE

Molecular formula. $C_4H_2FeO_4$

Relative molecular mass. 169.9

Graphic formula.



Chemical name. Iron(2+) fumarate (1 : 1); iron(2+) (*E*)-2-butenedioate (1 : 1); CAS Reg. No. 141-01-5.

Description. A fine, reddish orange or reddish brown powder.

Solubility. Slightly soluble in water; very slightly soluble in ethanol (~750 g/l) TS.

Category. Iron supplement.

Storage. Ferrous fumarate should be kept in a well-closed container.

Requirements

Definition. Ferrous fumarate contains not less than 93.0% and not more than 101.0% of $C_4H_2FeO_4$, calculated with reference to the dried substance.

Identity tests

A. Dissolve with heating 0.4 g in 10 ml of hydrochloric acid (1 mol/l) VS and cool. (Keep 1.0 ml of this solution for test B.) To the remaining solution add 15 ml of sodium hydroxide (1 mol/l) VS. Separate the dark precipitate by filtration. To the filtrate add 0.2 ml of phenolphthalein/ethanol TS and

sufficient hydrochloric acid (1 mol/l) VS until the pink colour disappears. To 2.0 ml of the resulting solution add 2.0 ml of copper(II) acetate (45 g/l) TS; a white, crystalline precipitate is produced.

B. The solution prepared in test A yields reaction A described under 2.1 General identification tests as characteristic of ferrous salts.

C. Mix 0.5 g with 1 g of resorcinol R. Transfer 0.5 g of the mixture to a crucible, add about 0.15 ml of sulfuric acid (~1760 g/l) TS and heat gently; a deep red, semi-solid mass is produced. Add the mass to a large volume of water; an orange-yellow solution is obtained which exhibits no fluorescence.

Heavy metals. Ignite 1.0 g gently until free from carbon, dissolve in 5 ml of hydrochloric acid (~420 g/l) TS by heating on a water-bath, and evaporate to dryness. Dissolve the residue in a mixture of 15 ml of hydrochloric acid (~420 g/l) TS, 4 ml of nitric acid (~1000 g/l) TS, and 6 ml of water, boil gently for 1 minute, cool, and extract the iron with 3 portions of ether R, each of 20 ml. Make a fourth extraction with a further 20 ml of ether R if the acid layer is more than slightly yellow and reject the extracts. Heat the acid solution gently to remove the ether, add 1 g of citric acid PbR, make alkaline with ammonia (~100 g/l) PbTS, add 1 ml of potassium cyanide PbTS and dilute to 40 ml with water; proceed to determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 100 µg/g.

Arsenic. Mix 0.2 g with 1.5 g of anhydrous sodium carbonate R, add 10 ml of bromine AsTS and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 20 ml of brominated hydrochloric acid AsTS and 10 ml of water. Transfer to a small flask, add sufficient stannous chloride AsTS to remove the yellow colour, connect to a condenser and distil 22 ml; proceed with the distillate as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 5 µg/g.

Ferric iron. Dissolve about 3 g, accurately weighed, in a mixture of 100 ml of water and 10 ml of hydrochloric acid (~420 g/l) TS by heating to boiling until dissolved. Cool rapidly and add 3 g of potassium iodide R, stopper the flask, swirl to mix, and allow to stand in the dark for 15 minutes. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, added towards the end of the titration. Repeat the operation without the substance being examined and determine the difference in the volume of sodium thiosulfate (0.1 mol/l) VS required for the titration. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 5.585 mg of ferric iron; the ferric iron content is not more than 20 mg/g.

Sulfates. Boil 0.15 g with 8 ml of hydrochloric acid (~70 g/l) TS and 20 ml of water, cool in ice and filter; proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 2 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Assay. Dissolve about 0.3 g, accurately weighed, in 7.5 ml of sulfuric acid (~100 g/l) TS, heating gently. Cool, add 25 ml of water, and immediately titrate with ceric ammonium sulfate (0.1 mol/l) VS, using 0.1 ml of *o*-phenanthroline TS as indicator. Each ml of eerie ammonium sulfate (0.1 mol/l) VS is equivalent to 16.99 mg of $C_4H_2FeO_4$.

FERROSI SULFAS
FERROUS SULFATE

Ferrous sulfate, exsiccated
Ferrous sulfate heptahydrate

Molecular formula. $FeSO_4,nH_2O$ (exsiccated); $FeSO_4, 7H_2O$ (heptahydrate).

Relative molecular mass. 151.9 (anhydrous); 278.0 (heptahydrate).

Chemical name. Iron (2+) sulfate (1 : 1); CAS Reg. No, 7720-78-7 (anhydrous). Iron (2+) sulfate (1 : 1) heptahydrate; CAS Reg. No. 7782-63-0 (heptahydrate).

Description. Exsiccated Ferrous sulfate is a greyish white powder; Ferrous sulfate heptahydrate has pale blue-green prisms or is a pale green, crystalline powder; both forms are odourless.

Solubility. Exsiccated Ferrous sulfate is slowly but almost completely soluble in carbon-dioxide-free water R; practically insoluble in ethanol (~750 g/l) TS. Ferrous sulfate heptahydrate is freely soluble in water; very soluble in boiling water; practically insoluble in ethanol (~750 g/l) TS.

Category. Haemopoietic (iron-deficiency anaemia).

Storage. Ferrous sulfate should be kept in a well-closed container.

Labelling. The designation on the container of Ferrous sulfate should state whether the substance is in the exsiccated form or is the heptahydrate.

Additional information. Exsiccated Ferrous sulfate is Ferrous sulfate deprived of part of its water of crystallization by drying at a temperature of 40 °C. Ferrous sulfate heptahydrate is efflorescent in dry air. Even in the absence of light, Ferrous sulfate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. The

crystals rapidly oxidize becoming brown. Both forms have a metallic and astringent taste.

Requirements

Definition. Exsiccated Ferrous sulfate contains not less than 80.0% and not more than 90.0% of $FeSO_4$. Ferrous sulfate heptahydrate contains not less than 98.0% and not more than 105.0% of $FeSO_4,7H_2O$.

Identity tests

A. A 20 mg/ml solution yields the reactions described under 2.1 General identification tests as characteristic of ferrous salts.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Dissolve 1.0 g in 10 ml of hydrochloric acid (~250 g/l) TS, add 2 ml of hydrogen peroxide (~330 g/l) TS, and evaporate to 5 ml. Allow to cool, dilute to 20 ml with hydrochloric acid (~250 g/l) TS, transfer the solution to a separating funnel and shake for 3 minutes with 3 successive quantities, each of 20 ml, of a solution composed of 100 ml of freshly distilled methylisobutylketone R shaken with 1 ml of hydrochloric acid (~250 g/l) TS. Allow the layers to separate, evaporate the aqueous layer to half its volume, allow to cool and dilute to 50 ml with water. Neutralize 25 ml to litmus TS and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A, multiplying the result by a factor of 2; not more than 50 µg/g.

Alkaline salts. Dissolve 1 g in 10 ml of water and oxidize by warming with a few drops of nitric acid (~1000 g/l) TS, make alkaline with ammonia (~100 g/l) TS, filter, evaporate the filtrate, ignite the residue and weigh; not more than 1.0 mg/g.

Arsenic. Use a solution of 3.3 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Insoluble matter. Dissolve 1.0 g in 10 ml of water, filter, wash the filter with water and dry it at 105 °C; the content of insoluble matter is not more than 5.0 mg/g.

pH value. pH of a 0.05 g/ml solution, 3.0–4.0.

Assay. For exsiccated Ferrous sulfate dissolve about 0.3 g, accurately weighed, in a mixture of 30 ml of water and 20 ml of sulfuric acid (~100 g/l) TS and titrate with ceric ammonium sulfate (0.1 mol/l) VS, using 2 drops of *o*-phenanthroline

TS as indicator. Each ml of ceric ammonium sulfate (0.1 mol/l) VS is equivalent to 15.19 mg of $FeSO_4$.

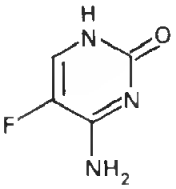
For Ferrous sulfate heptahydrate dissolve 2.5 g of sodium hydrogen carbonate R in a mixture of 150 ml of water, 10.0 ml of sulfuric acid (~1760 g/l) TS, and 5.0 ml of phosphoric acid (~1440 g/l) TS. When the effervescence ceases, add about 0.5 g of the test substance, accurately weighed, and when solution is complete titrate with ceric ammonium sulfate (0.1 mol/l) VS, using 2 drops of *o*-phenanthroline TS as indicator. Each ml of ceric ammonium sulfate (0.1 mol/l) VS is equivalent to 27.80 mg of $FeSO_4,7H_2O$.

FLUCYTOSINUM
FLUCYTOSINE

Molecular formula. $C_4H_4FN_3O$

Relative molecular mass. 129.1

Graphic formula.



Chemical name. 5-Fluorocytosine; 4-amino-5-fluoro-2(1*H*)-pyrimidinone; CAS Reg. No. 2022-85-7.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antifungal drug.

Storage. Flucytosine should be kept in a tightly closed container, protected from light.

Additional information. Flucytosine melts at about 295 °C.

Requirements

Definition. Flucytosine contains not less than 98.5% and not more than 101.0% of $C_4H_4FN_3O$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from flucytosine RS or with the *reference spectrum* of flucytosine.

B. The absorption spectrum of a 5.0 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 286 nm; the absorbance of a 1-cm layer at this wavelength is about 0.36.

C. See the test described below under "Fluorouracil". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

D. Dissolve 0.05 g in 5 ml of water and add 0.15 ml of bromine TS1; the colour is discharged or almost discharged.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

Fluorouracil. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 70 volumes of nitromethane R, 20 volumes of ethanol (~750 g/l) TS, and 10 volumes of lithium chloride (10 g/l) TS as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a solvent mixture composed of 15 volumes of methanol R and 10 volumes of water, containing (A) 10 mg of the test substance per ml and (B) 10 mg of flucytosine RS per ml; then apply also 10 µl of each of the 2 following solutions in the above solvent mixture containing (C) 20 mg of the test substance per ml and (D) 20 µg of fluorouracil RS per ml. Develop the plate in an unsaturated chromatographic chamber. After removing the plate from the chromatographic chamber, allow it to dry in a current of air and examine the

chromatogram in ultraviolet light (254 nm). The spot obtained with solution D is more intense than any corresponding spot obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in a mixture of 50 ml of acetic anhydride R and 100 ml of glacial acetic acid R1 with warming, if necessary, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 12.91 mg of $C_4H_4FN_3O$.

Additional requirement for Flucytosine for parenteral use

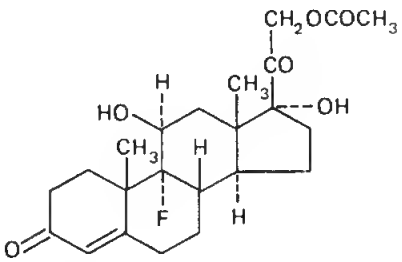
Complies with the monograph for "Parenteral preparations".

FLUDROCORTISONI ACETAS
FLUDROCORTISONE ACETATE

Molecular formula. $C_{23}H_{31}FO_6$

Relative molecular mass. 422.5

Graphic formula.



Chemical name. 9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate; 21-(acetyloxy)-9-fluoro-11β,17-dihydroxypregn-4-ene-3,20-dione; CAS Reg. No. 514-36-3.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Adrenal hormone.

Storage. Fludrocortisone acetate should be kept in a well-closed container, protected from light.

Additional information. Fludrocortisone acetate is hygroscopic.

Requirements

Definition. Fludrocortisone acetate contains not less than 96.0% and not more than 104.0% of $C_{23}H_{31}FO_6$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from fludrocortisone acetate RS or with the *reference spectrum* of fludrocortisone acetate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours and carry out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 75 volumes of toluene R and 25 volumes of chloroform R. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of fludrocortisone acetate RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat it at 120 °C for 15 minutes, spray it with sulfuric acid/ethanol TS, and then heat it at 120 °C for 10 minutes. Allow it to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Heat 0.5 ml of chromic acid TS in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube but there is no greasiness. Add about 3 mg of the test substance and again heat in a water-bath for 5 minutes; the solution no longer wets the sides of the tube.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +148$ to $+156°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Ultraviolet absorption. Absorbance of a 1-cm layer of a 10 µg/ml solution in dehydrated ethanol R at about 240 nm; 0.39–0.42.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R2 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R, and 0.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber allow it to dry in air until the solvents have evaporated; then heat it at 105 °C for 10 minutes, allow it to cool, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

- The solutions must be protected from light throughout the assay.

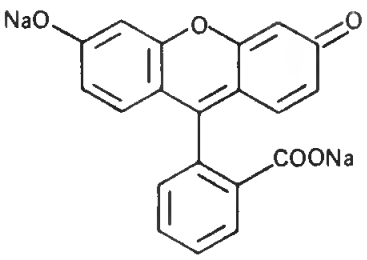
Dissolve about 25 mg, accurately weighed, in sufficient aldehyde-free ethanol (~750 g/l) TS to produce 250 ml. Dilute 10 ml of this solution with sufficient aldehyde-free ethanol (~750 g/l) TS to produce 50 ml. Transfer 10.0 ml of the diluted solution to a 25-ml volumetric flask, add 2.0 ml of blue tetrazolium/ethanol TS, and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner. Calculate the amount of $C_{23}H_{31}FO_6$ in the substance being tested by comparison with fludrocortisone acetate RS, similarly and concurrently examined.

FLUORESCEINUM NATRICUM
FLUORESCEIN SODIUM

Molecular formula. $C_{20}H_{10}Na_2O_5$

Relative molecular mass. 376.3

Graphic formula.



Chemical name. Fluorescein disodium salt; 2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid disodium salt; 3′,6′-dihydroxyspiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthene]-3-one disodium salt; CAS Reg. No. 518-47-8.

Description. An orange-red powder; odourless.

Solubility. Soluble in 1.5 parts of water; soluble in ethanol (~750 g/l) TS.

Category. Diagnostic agent in ophthalmology.

Storage. Fluorescein sodium should be kept in a well-closed container, protected from light.

Additional information. Fluorescein sodium is hygroscopic.

Requirements

Definition. Fluorescein sodium contains not less than 98.0% and not more than 100.5% of $C_{20}H_{10}Na_2O_5$, calculated with reference to the dried substance.

Identity tests

A. A solution in water is strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acid and reappears when it is made alkaline.

B. Ignite 20 mg and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

C. Dissolve 1 mg in 2 ml of water, place 0.05 ml of this solution on a piece of filter-paper; a yellow spot is produced. Expose the moist paper to the vapour of bromine R for 1 minute and then to the vapour of ammonia (~260 g/l) TS; the yellow colour of the spot changes to deep pink.

Chlorides. Dissolve 0.07 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 3.5 mg/g.

Sulfates. Dissolve 0.05 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 10 mg/g.

Zinc. Dissolve 0.10 g in 10 ml of water, add 2 ml of hydrochloric acid (~420 g/l) TS, filter, and add 0.1 ml of potassium ferrocyanide (45 g/l) TS; no turbidity or precipitate is produced immediately.

Chloroform-soluble matter. Dissolve 0.20 g in 10 ml of sodium hydroxide (0.1 mol/l) VS and extract with 10 ml of chloroform R. Allow to separate, dry the chloroform layer over anhydrous sodium sulfate R, and filter. Measure the absorbance of the filtrate in a 1-cm cell at a maximum of 480 nm against a solvent cell containing chloroform R; the absorbance does not exceed 0.10.

Ethanol-insoluble matter. Boil 0.2 g with 20 ml of ethanol (~750 g/l) TS for 1 minute, filter through a sintered glass filter, wash the filter with ethanol (~750 g/l) TS until the filtrate is almost colourless, dry the filter at 105 °C for 1 hour and weigh; the residue is not more than 2.0 mg.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 100 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 7.0–9.0.

Dimethylformamide. Carry out the test as described under 1.14.5 Gas chromatography. Prepare an internal standard consisting of a mixture of 20 µl of dimethylacetamide R in 100 ml of water. Inject the following 3 solutions: (1) a mixture of 2 µl of dimethylformamide R in 10 ml of water and containing 10 ml of the solution of the internal standard; (2) for the determination of the retention time of the substance being examined, dissolve 1.0 g of the test substance in 10 ml of water, add with stirring 10 ml of hydrochloric acid (0.5 mol/l) VS, allow to stand for 15 minutes, centrifuge, and then dissolve 0.10 g of trisodium orthophosphate R in 5 ml of the supernatant liquid; (3) dissolve 1.0 g of the test substance in 10 ml of the solution of the internal standard, add with stirring 10 ml of hydrochloric acid (0.5 mol/l) VS, allow to stand for 15 minutes, centrifuge, and then dissolve 0.10 g of trisodium orthophosphate R in 5 ml of the supernatant liquid.

For the procedure, use a glass column 1.5 m long and 4 mm in internal diameter packed with an adequate quantity of an adsorbent composed of 1 g of macrogol 1000 R supported on 9 g of acid-washed, silanized diatomaceous support R and maintained at 120 °C. Use nitrogen R as the carrier gas and a flame ionization detector.

In the chromatogram obtained with solution 3, the ratio of the area of any peak due to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio for the chromatogram obtained with solution 1.

Resorcinol. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 6 volumes of hexane R and 4 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of the 2 following solutions: (A) dissolve 1.0 g of the test substance in 10 ml of water, add slowly with constant stirring 10 ml of hydrochloric acid (0.5 mol/l) VS, allow to stand for 15 minutes, centrifuge, and use the supernatant liquid; (B) dissolve 2.5 mg of resorcinol R in 10 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air, and expose it to the vapour of iodine R for 30 minutes. Examine the chromatogram in daylight. The spot obtained with solution B is more intense than any corresponding spot obtained with solution A.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 8 volumes of chloroform R and 2 volumes of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in hydrochloric acid/methanol (0.1 mol/l) VS containing (A) 10 mg of the test substance per ml and (B) 20 µg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and expose the plate to the vapour of iodine R for 30 minutes. Examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 20 ml of water, add 5 ml of hydrochloric acid (~70 g/l) TS, and extract with 4 volumes, each of 20 ml, of a solvent mixture composed of equal volumes of 2-butanol R and chloroform R. Separate and combine the extracts, wash with 10 ml of water, extract the washings with 5 ml of the above solvent mixture, and add to the combined extracts. Evaporate the mixed extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of ethanol (~750 g/l) TS, evaporate to dryness on a water-bath and dry to constant weight at 105 °C. Each g of residue is equivalent to 1.132 g of $C_{20}H_{10}Na_2O_5$.

Additional requirement for Fluorescein sodium for sterile use

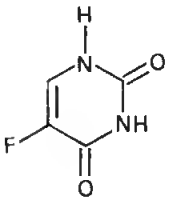
Complies with 3.2.1 Test for sterility of non-injectable preparations.

FLUOROURACILUM
FLUOROURACIL

Molecular formula. $C_4H_3FN_2O_2$

Relative molecular mass. 130.1

Graphic formula.



Chemical name. 5-Fluorouracil; 5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione; CAS Reg. No. 51-21-8.

Description. A white or almost white, crystalline powder.

Solubility. Sparingly soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Fluorouracil should be kept in a tightly closed container, protected from light.

Additional information. Fluorouracil melts at about 282 °C with decomposition. CAUTION: Fluorouracil must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Fluorouracil contains not less than 98.5% and not more than 101.0% of $C_4H_3FN_2O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from fluorouracil RS or with the *reference spectrum* of fluorouracil.

B. The absorption spectrum of a 10 µg/ml solution in acetate buffer, pH 4.7, TS, when observed between 220 nm and 350 nm, is qualitatively similar to that of a 10.0 µg/ml solution of fluorouracil RS in acetate buffer, pH 4.7, TS (a maximum occurs at about 266 nm and a minimum occurs at about 232 nm). The absorbances of the solutions at the maximum do not differ from each other by more than 3%. The absorbance of a 1-cm layer at 266 nm is about 0.54.

C. Heat 0.5 ml of chromic acid TS in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube but there is no greasiness. Add about 3 mg of the test substance arid again heat in a water-bath for 5 minutes; the solution no longer wets the sides of the tube.

D. Dissolve 0.05 g in 5 ml of water, add 1 ml of bromine TS1; the colour of the bromine is discharged.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 70 volumes of ethyl acetate R, 15 volumes of methanol R, and 15 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of equal volumes of water and methanol R containing (A) 20 mg of the test substance per ml and (B) 0.050 mg of fluorouracil RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Fluorine content. Carry out the combustion as described under 2.4 Oxygen flask method, using 7 mg of the test substance, and adding about 15 mg of sodium peroxide R and 15 ml of sodium hydroxide (0.1 mol/l) VS as the absorbing liquid. When the process is complete, allow the flask to stand for not less than 10 minutes with intermittent shaking, then dilute the contents to 100 ml with water. Proceed with 5.0 ml as described under 2.4 Oxygen flask method for the determination of fluorine; not more than 55 µg of F.

Assay. Dissolve about 0.4 g, accurately weighed, in 80 ml of dimethylformamide R, add 0.25 ml of thymol blue/dimethylformamide TS and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS to a blue endpoint as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 13.01 mg of $C_4H_3FN_2O_2$.

Additional requirements for Fluorouracil for parenteral use

Complies with the monograph for "Parenteral preparations".

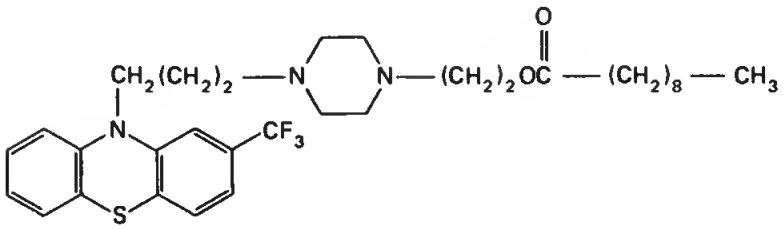
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.33 IU of endotoxin RS per mg.

FLUPHENAZINI DECANOAS
FLUPHENAZINE DECANOATE

Molecular formula. $C_{32}H_{44}F_3N_3O_2S$

Relative molecular mass. 591.8

Graphic formula.



Chemical name. 4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol decanoate (ester); 4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol decanoate (ester); CAS Reg. No. 5002-47-1.

Description. A pale yellow viscous liquid or a yellow, crystalline, oily solid; odour, faint, ester-like.

Miscibility. Immiscible with water; miscible with dehydrated ethanol R and ether R.

Category. Neuroleptic.

Storage. Fluphenazine decanoate should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Fluphenazine decanoate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Fluphenazine decanoate contains not less than 98.5% and not more than 101.5% of $C_{32}H_{44}F_3N_3O_2S$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from fluphenazine decanoate RS or with the *reference spectrum* of fluphenazine decanoate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of *n*-tetradecane R and 95 volumes of hexane R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached the top of the plate, remove the plate from the chromatographic chamber and allow to stand at room temperature until the solvents have completely evaporated. Use the impregnated plate immediately, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 90 volumes of methanol R and 10 volumes of water. Apply separately to the plate 1 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 20 mg of the test substance per ml and (B) 20 mg of fluphenazine decanoate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 5 mg in 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a reddish brown colour is produced.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of acetone R, 30 volumes of cyclohexane R and 5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 20 µl of each of 2 solutions in methanol R containing (A) 25 mg of the test substance per ml and (B) 0.25 mg of fluphenazine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Then spray the plate with sulfuric acid (~635 g/l) TS and examine the chromatogram in daylight. Using either method of visualization, any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.6 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.59 mg of $C_{32}H_{44}F_3N_3O_2S$.

Additional requirement for Fluphenazine decanoate for parenteral use

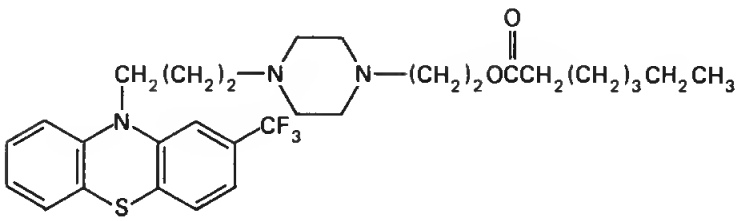
Complies with the monograph for "Parenteral preparations".

FLUPHENAZINI ENANTAS
FLUPHENAZINE ENANTATE

Molecular formula. $C_{29}H_{38}F_3N_3O_2S$

Relative molecular mass. 549.7

Graphic formula.



Chemical name. 4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol heptanoate (ester); 4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol heptanoate (ester); CAS Reg. No. 2746-81-8.

Description. A pale yellow, viscous liquid or a yellow, crystalline, oily solid; odour, faint, ester-like.

Miscibility. Immiscible with water; miscible with dehydrated ethanol R, and ether R.

Category. Neuroleptic.

Storage. Fluphenazine enantate should be kept in a well-closed container, protected from light.

Requirements

Definition. Fluphenazine enantate contains not less than 98.5% and not more than 101.5% of $C_{29}H_{38}F_3N_3O_2S$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from fluphenazine enantate RS or with the *reference spectrum* of fluphenazine enantate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of *n*-tetradecane R and 95 volumes of hexane R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached the top of the plate, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvents have completely evaporated. Use the impregnated plate immediately, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 90 volumes of methanol R and 10 volumes of water. Apply separately to the plate 1 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 20 mg of the test substance per ml and (B) 20 mg of fluphenazine enantate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 5 mg in 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a reddish brown colour is produced.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of acetone R, 30 volumes of cyclohexane R, and 5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 20 µl of each of 2 solutions in methanol R containing (A) 25 mg of the test substance per ml and (B) 0.25 mg of fluphenazine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Then spray the plate with sulfuric acid (~635 g/l) TS and examine the chromatogram in daylight. Using either method of visualization, any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.49 mg of $C_{29}H_{38}F_3N_3O_2S$.

Additional requirement for Fluphenazine enantate for parenteral use

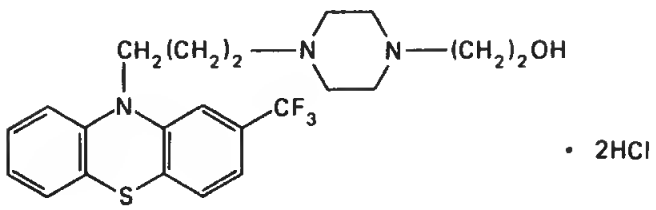
Complies with the monograph for "Parenteral preparations".

FLUPHENAZINI HYDROCHLORIDUM
FLUPHENAZINE HYDROCHLORIDE

Molecular formula. $C_{22}H_{26}F_3N_3OS,2HCl$

Relative molecular mass. 510.4

Graphic formula.



Chemical name. 4-[3-[2-(Trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride; 4-[3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride; CAS Reg. No. 146-56-5.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 10 parts of water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Neuroleptic.

Storage. Fluphenazine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Fluphenazine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Fluphenazine hydrochloride contains not less than 98.5% and not more than 101.5% of $C_{22}H_{26}F_3N_3OS,2HCl$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from fluphenazine hydrochloride RS or with the *reference spectrum* of fluphenazine hydrochloride.

B. Carry out the test in subdued light as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 15 volumes of formamide R, 5 volumes of 2-phenoxyethanol R, and 180 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached the top of the plate, remove the plate from the chromatographic chamber and allow to stand at room temperature until the solvents have completely evaporated. Use the impregnated plate immediately, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 2 volumes of diethylamine R and 100 volumes of light petroleum R1 saturated with 2-phenoxyethanol R. Apply separately to the plate 2 µl of each of 2 solutions in chloroform R containing (A) 2.0 mg of the test substance per ml and (B) 2.0 mg of fluphenazine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm), observing

the fluorescence produced after about 2 minutes. Spray the plate with sulfuric acid/ethanol TS and examine the chromatogram in daylight. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 5 mg in 5 ml of sulfuric acid (~1760 g/l) TS; an orange colour is produced which becomes brownish red on warming.

D. Heat 0.5 ml of chromic acid TS in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube but there is no greasiness. Add about 3 mg of the test substance and again heat in a water-bath for 5 minutes; the solution no longer wets the sides of the tube.

E. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of acetone R, 30 volumes of cyclohexane R and 5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in sodium hydroxide/methanol TS containing (A) 10 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

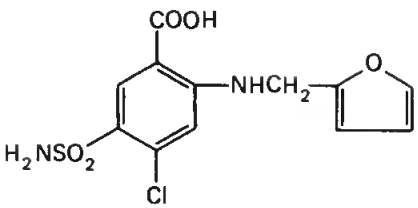
Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.52 mg of $C_{22}H_{26}F_3N_3OS,2HCl$.

FUROSEMIDUM
FUROSEMIDE

Molecular formula. $C_{12}H_{11}ClN_2O_5S$

Relative molecular mass. 330.8

Graphic formula.



Chemical name. 4-Chloro-*N*-furfuryl-5-sulfamoylanthranilic acid; 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; CAS Reg. No. 54-31-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 75 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Diuretic.

Storage. Furosemide should be kept in a well-closed container, protected from light.

Requirements

Definition. Furosemide contains not less than 98.0% and not more than 101.0% of $C_{12}H_{11}ClN_2O_5S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from furosemide RS or with the *reference spectrum* of furosemide.

B. Dissolve 5 mg in 10 ml of methanol R. Transfer 1 ml of this solution to a flask, add 10 ml of hydrochloric acid (~70 g/l) TS and heat under a reflux condenser for 15 minutes. Cool and add 15 ml of sodium hydroxide (1 mol/l) VS and 5 ml of sodium nitrite (1 g/l) TS. Allow to stand for 3 minutes, then add 2 ml of ammonium sulfamate (25 g/l) TS and mix. Add 1 ml of *N*-(1-naphthyl) ethylenediamine hydrochloride (5 g/l) TS; a red-violet colour is produced.

C. Dissolve 25 mg in 2.5 ml of ethanol (~750 g/l) TS and add, drop by drop, about 2 ml of 4-dimethylaminobenzaldehyde TS1; a transient green colour is produced, which becomes deep red.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

4-Chloro-5-sulfamoylanthranilic acid. Dissolve 0.1 g in 25 ml of methanol R. To 1 ml add 3 ml of dimethylformamide R, 12 ml of water, and 1 ml of hydrochloric acid (1 mol/l) VS. Cool, add 0.5 ml of sodium nitrite (10 g/l) TS, shake, and allow to stand for 5 minutes. Add 1 ml of ammonium sulfamate (25 g/l) TS, shake, and allow to stand for 3 minutes. Add 1 ml of *N*-(1-naphthyl) ethylenediamine hydrochloride (5 g/l) TS and sufficient water to produce 25 ml. Measure the absorbance of a 1-cm layer of the resulting solution at a maximum of about 530 nm, using as a blank a solution prepared by treating a mixture of 1 ml of methanol R and 3 ml of dimethylformamide R in a similar manner; the absorbance is not greater than 0.12 (0.3% of free primary aromatic amines expressed as 4-chloro-5-sulfamoylanthranilic acid) (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 1 volume of toluene R, 1 volume of xylene R, 3 volumes of dioxan R, 3 volumes of 2-propanol R and 2 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in acetone R containing (A) 20 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 40 ml of dimethylformamide R, add 3 drops of bromothymol blue/dimethylformamide TS, and titrate with sodium hydroxide (0.1 mol/l) VS to a blue endpoint. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 33.08 mg of $C_{12}H_{11}ClN_2O_5S$.

Additional requirements for Furosemide for parenteral use

Complies with the monograph for "Parenteral preparations".

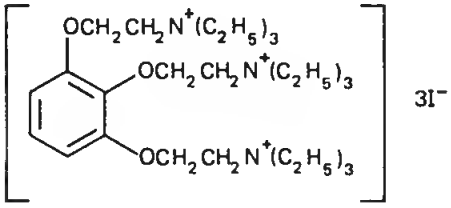
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 3.6 IU of endotoxin RS per mg.

GALLAMINI TRIETHIODIDUM
GALLAMINE TRIETHIODIDE

Molecular formula. $C_{30}H_{60}I_3N_3O_3$

Relative molecular mass. 891.5

Graphic formula.



Chemical name. [*v*-Phenenyltris(oxyethylene)]tris[triethylammonium] triiodide; 2,2′,2″-[1,2,3-benzenetriyltris(oxy)]tris[*N,N,N*-triethylethanaminium triiodide; 1,2,3-tris(2-diethylaminoethoxy)benzene triethiodide; CAS Reg. No. 65-29-2.

Description. A white or almost white powder; odourless.

Solubility. Very soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Muscle relaxant.

Storage. Gallamine triethiodide should be kept in a tightly closed container, protected from light.

Additional information. Gallamine triethiodide is hygroscopic.

Requirements

Definition. Gallamine triethiodide contains not less than 98.0% and not more than 101.0% of $C_{30}H_{60}I_3N_3O_3$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from gallamine triethiodide RS or with the *reference spectrum* of gallamine triethiodide.

B. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 220 nm and 350 nm exhibits a maximum at about 225 nm; the absorbance of a 1-cm layer at this wavelength is between 0.50 and 0.55.

C. Dissolve 0.05 g in 5 ml of water and add 1 ml of potassio-mercuric iodide TS; a yellow precipitate is produced.

D. A 0.01 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of iodides.

Clarity and colour of solution. A freshly prepared solution of 0.20 g in 10 ml of carbon-dioxide free water R is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

Acidity or alkalinity. To 50 ml of water add 0.2 ml of methyl red/ethanol TS and adjust to pH 6 by adding either sulfuric acid (0.01 mol/l) VS or sodium hydroxide (0.02 mol/l) VS until the colour is orange-yellow. Add 1.0 g of the substance being examined and shake to dissolve; not more than 0.2 ml of either sulfuric acid (0.01 mol/l) VS or sodium hydroxide (0.02 mol/l) VS is required to restore the original orange-yellow colour.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R1 as the coating substance and a mixture of 17 volumes of glacial acetic acid R, 17 volumes of water and 66 volumes of 1-butanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 5.0 mg of the test substance per ml and (B) 0.05 mg of the test substance per ml. Allow the mobile phase to ascend 10 cm. After removing the plate from the chromatographic chamber, dry it in a current of warm air and spray it with potassium iodoplatinate TS. An elongated blue spot, which may appear to be double, is obtained on the chromatogram from test solution A. Any spot above the principal spot obtained with solution A is not more intense than the principal spot obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 40 ml of acetone R, and add 15 ml of mercuric acetate/acetic acid TS. Titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 29.72 mg of $C_{30}H_{60}I_3N_3O_3$.

GELATINA
GELATIN

Chemical name. Gelatin; CAS Reg. No. 9000-70-8.

Description. Faintly yellow to amber-coloured sheets, flakes, granules, or powder; practically odourless; in solution it has a slight, characteristic, bouillon-like odour.

Solubility. Practically insoluble in most organic solvents. In cold water it swells and softens, absorbing 5–10 times its own mass of water. After swelling, soluble in hot water, in acetic acid (~300 g/l) TS, and in a hot mixture of glycerol R and water.

Category. Encapsulating agent; tablet binder; coating agent; suspending agent; viscosity-increasing agent.

Storage. Gelatin should be kept in a well-closed container.

Additional information. These specifications do not necessarily apply to gelatin for parenteral use or other particular application. Attention should be paid to the microbiological quality since gelatin is of natural origin.

The type of gelatin may be distinguished by the following test:

Dissolve 1 g in 100 ml of hot water. Place aliquots of 5 ml into six separate test-tubes and add 5 ml of a buffer to each tube, using buffers of pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 (citrate buffer, pH 4.0, TS, phosphate buffer, pH 4.0, TS, or phthalate buffer, pH 4.0, TS; acetate buffer, pH 5.0, TS; phosphate/citrate buffer, pH 6.0, TS or acetate buffer, pH 6.0, TS; phosphate buffer, pH 7.0, TS; phosphate buffer, pH 8.0, TS or buffer borate, pH 8.0, TS; buffer borate, pH 9.0, TS). Cool the test-tubes and allow them to stand at 4 °C for 24 hours; the type of gelatin is recognized by the resulting opalescence – a maximum opalescence appearing at pH 5.0 indicates gelatin type B, while a maximum opalescence between pH 7.0 and pH 9.0 indicates gelatin type A.

Requirements

Definition. Gelatin is a purified protein obtained either by the partial acid hydrolysis (type A) or by the partial alkali hydrolysis (type B) of animal collagen. It can exist as a mixture of both types.

Identity tests

A. Dissolve 1 g in carbon-dioxide-free water R, heat to about 55 °C, and dilute to 100 ml with the same solvent. Keep the solution at this temperature

throughout the following test (retain the solution for test C): to 2 ml add 0.05 ml of copper(II) sulfate (160 g/l) TS, mix, and add 0.5 ml of sodium hydroxide (~80 g/l) TS; a violet colour is produced.

B. Transfer 0.5 g to a test-tube, add 10 ml of water, and allow to stand for 10 minutes. Heat at 60 °C for 15 minutes and keep the tube in a vertical position at 0 °C for 6 hours. Invert the tube; the content does not immediately flow out.

C. Acidify 2 ml of the solution prepared for test A and add 0.5 ml of potassium dichromate (100 g/l) TS; a yellow precipitate is formed.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Arsenic. Use a solution of 1.0 g in a mixture of 2.5 ml of sulfuric acid (~1760 g/l) TS, 2.5 ml of nitric acid (~1000 g/l) TS, and a slight excess of bromine TS1, allow to stand for 30 minutes, and boil under a reflux condenser for 1 hour. Proceed with the test as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 1 µg/g.

Odour and water-insoluble substances. Dissolve 1 g in 40 ml of hot water; no disagreeable odour is perceptible. Observe the solution through a layer of 2 cm; only a slight opalescence appears.

Sulfated ash. Use 2.0 g; not more than 30 mg/g.

Loss on drying. Weigh 10 g and dry to constant mass at 105 °C; it loses not more than 150 mg/g.

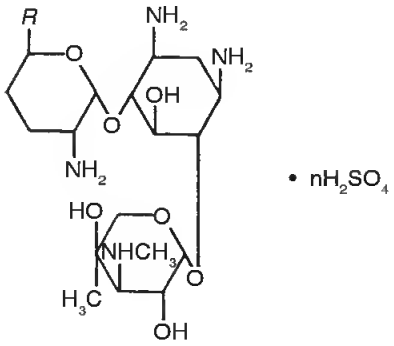
Sulfur dioxide. Dissolve 20 g in 150 ml of hot water using a round-bottom flask with a long neck. Add 5 ml of phosphoric acid (~1440 g/l) TS and 1 g of sodium hydrogen carbonate R, and without delay connect the flask to a condenser. (*Note*. Excessive foaming can be reduced by adding a few drops of an antifoaming agent.) Distil 50 ml, allowing the distillate to be collected under a 50 ml surface of iodine (0.05 mol/l) VS. Acidify the distillate with a few drops of hydrochloric acid (~70 g/l) TS, add 2 ml of barium chloride (50 g/l) TS, and heat on a water-bath until the liquid is nearly colourless. If any, filter the precipitated barium sulfate, wash, ignite, and weigh. Repeat the procedure without the Gelatin being examined and make any necessary corrections. The content of barium sulfate is not more than 109.3 mg, which corresponds to not more than 1.5 mg/g of sulfur dioxide.

GENTAMICINI SULFAS
GENTAMICIN SULFATE

Gentamicin sulfate (non-injectable)
Gentamicin sulfate, sterile

Chemical name. Gentamicin sulfate; CAS Reg. No. 1405-41-0.

Graphic formula.



Gentamicin	R
C_1	$H_3C—HN—C(CH_3)—H$
C_2	$H_2N—C(CH_3)—H$
C_{1a}	CH_2NH_2

Description. A white to cream-coloured powder; odourless.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS, and ether R.

Category. Antibacterial drug.

Storage. Gentamicin sulfate should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Gentamicin sulfate indicates that the substance complies with the additional requirements for sterile Gentamicin sulfate and may be used for parenteral administration or for other sterile applications.

Additional information. Gentamicin sulfate is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Gentamicin sulfate is the sulfate salt of gentamicin fractions C_1, C_2, and C_{1a} produced by the growth of *Micromonospora purpurea.*

Gentamicin sulfate contains not less than 590 International Units of gentamicin per mg, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance (a precoated plate from a commercial source is suitable); shake together 1 volume of chloroform R, 1 volume of methanol R, and 1 volume of ammonia (~260 g/l) TS, allow to separate and use the lower layer as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions containing (A) 20 mg of the test substance per ml and (B) 20 mg of gentamicin sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with triketohydrindene/pyridine/acetone TS, and heat it at 105 °C for 2 minutes. Examine the chromatogram in daylight. The 3 principal spots obtained with solution A correspond with the 3 principal spots obtained with solution B.

B. A 10 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 0.10 g/ml solution, and calculate with reference to the anhydrous substance: $[\alpha]_D^{20\,°C}$ = +107 to +121 °.

Sulfated ash. Not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not more than 150 mg/g.

pH value. pH of a 40 mg/ml solution, 3.5–5.5.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either *Bacillus pumilus* (NCTC 8241; ATCC 14884), *Bacillus subtilis* (ATCC 6633), or *Staphylococcus aureus* (ATCC 6538P) as the test organism, culture medium Cm1 with a final pH of 7.8, sterile phosphate buffer pH 8.0 TS1 or TS2, an appropriate concentration of gentamicin (usually between 2 and 20 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 590 IU per mg, calculated with reference to the anhydrous substance.

Additional requirements for Gentamicin sulfate for sterile use

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

Additional requirements for Gentamicin sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

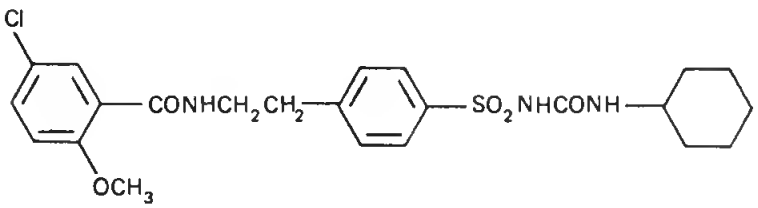
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.70 IU of endotoxin RS per mg of gentamicin.

GLIBENCLAMIDUM
GLIBENCLAMIDE

Molecular formula. $C_{23}H_{28}ClN_3O_5S$

Relative molecular mass. 494.0

Graphic formula.



Chemical name. 1-[[*p*-[2-(5-Chloro-*o*-anisamido)ethyl]phenyl]sulfonyl]-3-cyclohexylurea; 5-chloro-*N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]-ethyl]-2-methoxybenzamide; 1-[4-[2-(5-chloro-2-methoxybenzamido)ethyl]-phenylsulfonyl]-3-cyclohexylurea; CAS Reg. No. 10238-21-8.

Other name. Glyburide.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water and ether R; slightly soluble in ethanol (~750 g/l) TS and methanol R.

Category. Antidiabetic agent.

Storage. Glibenclamide should be kept in a well-closed container.

Requirements

Definition. Glibenclamide contains not less than 98.5% and not more than 101.0% of $C_{23}H_{28}ClN_3O_5S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from glibenclamide RS or with the *reference spectrum* of glibenclamide.

B. The absorption spectrum of a 0.10 mg/ml solution in hydrochloric acid/methanol (0.01 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 300 nm and a less intense maximum at about 275 nm; the absorbance of a 1-cm layer at 300 nm is about 0.63.

C. Melting temperature, about 172 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 45 volumes of chloroform R, 45 volumes of cyclohexane R, 5 volumes of ethanol (~750 g/l) TS, and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 10 mg of the test substance per ml and (B) 0.05 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 100 ml of hot neutralized ethanol TS, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using

phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary correction. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 49.40 mg of $C_{23}H_{28}ClN_3O_5S$.

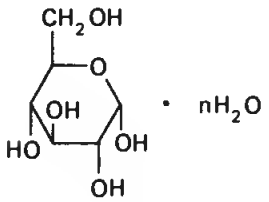
GLUCOSUM
GLUCOSE

Glucose, anhydrous
Glucose monohydrate

Molecular formula. $C_6H_{12}O_6$ (anhydrous); $C_6H_{12}O_6,H_2O$ (monohydrate).

Relative molecular mass. 180.2 (anhydrous); 198.2 (monohydrate).

Graphic formula.



n = 0 (anhydrous)
n = 1 (monohydrate)

Chemical name. α-D-Glucopyranose; CAS Reg. No. 492-62-6 (anhydrous). α-D-Glucopyranose monohydrate; CAS Reg. No. 14431-43-7 (monohydrate).

Other name. Dextrose.

Description. Colourless crystals or a white, crystalline or granular powder; odourless.

Solubility. Soluble in about 1 part of water; slightly soluble in ethanol (~750 g/l) TS; more soluble in boiling water and boiling ethanol (~750 g/l) TS.

Category. Nutrient; fluid replenisher.

Storage. Glucose should be kept in a well-closed container.

Labelling. The designation on the container of Glucose should state whether the substance is the monohydrate or is in the anhydrous form. If the material is not intended for parenteral use a designation "for oral use only" should be added.

Additional information. Glucose has a sweet taste.

Requirements

Definition. Glucose contains not less than 99.0% and not more than 101.5% of $C_6H_{12}O_6$, calculated with reference to the anhydrous substance.

Identity tests

A. When heated it melts, swells up and burns, evolving an odour of burnt sugar.

B. Add a few drops of a 0.05 g/ml solution to 5 ml of hot potassio-cupric tartrate TS; a copious red precipitate is produced.

Specific optical rotation. Dissolve 10.0 g in 50 ml of water, add 0.2 ml of ammonia (~100 g/l) TS, and sufficient water to produce 100 ml, and allow to stand for 30 minutes. Calculate the result with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +52.5$ to $+53.0°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 5 µg/g.

Arsenic. Use a solution of 10 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; not more than 1 µg/g.

Chlorides. Dissolve 1.25 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfates. Dissolve 2.5 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Less-soluble sugars and dextrins. Boil 1 g with 30 ml of ethanol (~710 g/l) TS and cool; a clear solution is produced.

Soluble starch. Dissolve 2.5 g in 25 ml of water, boil the solution for 1 minute, cool and add 0.1 ml of iodine (0.1 mol/l) VS; no blue colour is produced.

Sulfites. Dissolve 2.5 g in 25 ml of water, add 0.1 ml of iodine (0.1 mol/l) VS and a few drops of starch TS; a blue colour is produced.

Clarity and colour of solution. A solution of 5.0 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Gn3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method. Method A. For the anhydrous form use about 1 g of the substance; the water content is not more than 10 mg/g. For the monohydrate use about 0.15 g of the substance; the water content is not less than 70 mg/g and not more than 95 mg/g.

Acidity. Dissolve 5.0 g in 50 ml of carbon-dioxide-free water R and titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS, using phenolphthalein/ethanol TS as indicator; not more than 0.5 ml is required to obtain the midpoint of the indicator (pink).

Assay. Dissolve about 0.10 g, accurately weighed, in 50 ml of water, add 25.0 ml of iodine (0.05 mol/l) VS and 10 ml of sodium carbonate (50 g/l) TS. Allow to stand for 20 minutes in the dark and add 15 ml of sulfuric acid (~100 g/l) TS. Titrate the excess of iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of iodine (0.05 mol/l) VS is equivalent to 9.008 mg of $C_6H_{12}O_6$.

Additional requirements for Glucose for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.

GLYCEROLI MONOSTEARAS
GLYCERYL MONOSTEARATE

$C_{21}H_{42}O_4$

Chemical name. Monostearin; octadecanoic acid monoester with 1,2,3-propanetriol; CAS Reg. No. 31566-31-1.

Description. A white or yellowish white, hard waxy mass or unctuous powder or flakes; odourless or slight, agreeable, fatty odour.

Solubility. Practically insoluble in water; soluble in ether R, benzene R, and ethanol (~750 g/l) TS at 60 °C.

Category. Emulsifying agent; cream and ointment base.

Storage. Glyceryl monostearate should be kept in a tightly closed container, protected from light.

Additional information. Glyceryl monostearate may contain a suitable antioxidant. Self-emulsifying glyceryl monostearate contains additional emulsifying agents.

Requirements

Definition. Glyceryl monostearate is a mixture of mono-, di- and triglycerides of stearic and palmitic acids.

Glyceryl monostearate contains not less than the equivalent of **35.0%** of monoglycerides, calculated as $C_{20}H_{40}O_4$, and not more than the equivalent of **6.0%** of free glycerol.

Identity tests

A. Melting temperature, not lower than 55 °C.

B. Dip a strip of filter-paper in a freshly prepared solution containing 9.5 ml of sodium nitroprusside (8.5 g/l) TS and 0.5 ml of piperidine R. Place the moistened filter-paper into the evolving vapours over the mouth of the test-tube containing 1 g of Glyceryl monostearate and heat with 2 ml of phosphoric acid (~1440 g/l) TS; a deep blue colour develops on the paper.

C. Heat 2.5 g with 40 ml of potassium hydroxide/ethanol TS1 on a water-bath under reflux for 30 minutes. Add 30 ml of water, evaporate the ethanol, acidify the hot mixture with 15 ml of hydrochloric acid (~70 g/l) TS, cool, and shake with 50 ml of ether R. Wash the ether layer with three quantities, each of a mixture of 5 ml of sodium chloride (400 g/l) TS and 5 ml of water, dry the ether layer over anhydrous sodium sulfate R, and filter. Evaporate the filtrate and dry the residue under reduced pressure at room temperature. Melt the residue, introduce it into capillary tubes, and keep the tubes for 24 hours in a well-closed container. Melting temperature, not lower than 53 °C.

Acid value. Not more than 6.0.

Saponification value. 155–177.

Iodine value. Not more than 3.

Sulfated ash. Not more than 1.0 mg/g.

Assays. Transfer about 0.4 g, accurately weighed, to a glass-stoppered separating funnel, and dissolve in 50 ml of dichloromethane R. Add 25 ml of water and shake vigorously for 1 minute. Allow the layers to separate. If an emulsion is formed, add a few drops of glacial acetic acid R. Carry out the extraction three more times using 25 ml, 20 ml, and 20 ml of water. Combine the dichloromethane extracts and use them in the assay for *monoglycerides*. Filter the aqueous layers through a filter-paper moistened with water, wash the filter with two quantities, each of 5 ml, of water, and dilute the combined filtrates and wash liquids to 100 ml with water. Use this solution for the assay for *free glycerol*.

Free glycerol

Place 50 ml of the prepared aqueous solution in a 500-ml ground-glass-stoppered conical flask, add 25 ml of periodic-acetic acid TS, and shake cautiously. Allow to stand at a temperature between 25 and 30 °C for 30 minutes. Add 100 ml of water and 25 ml of potassium iodide (80 g/l) TS. Titrate with sodium thiosulfate (0.1 mol/l) VS, using 1 ml of starch TS as indicator added towards the end of the titration. Repeat the procedure without the Glyceryl monostearate being examined and make any necessary corrections.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 2.3 mg of glycerol.

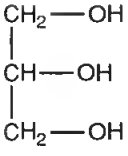
Monoglycerides

Filter the combined dichloromethane extracts through a plug of cotton-wool. Wash the separating funnel and the filter with three quantities, each of 5 ml, of dichloromethane R, and dilute the filtrate to 100 ml with dichloromethane R. Carry out the assay as described for free glycerol, using 50 ml of the dichloromethane solution.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 17.2 mg of monoglycerides, calculated as $C_{20}H_{40}O_4$.

GLYCEROLUM

GLYCEROL



$C_3H_8O_3$

Relative molecular mass. 92.09

Chemical name. Glycerol; 1,2,3-propanetriol; CAS Reg. No. 56-81-5.

Other name. Glycerin.

Description. A clear, colourless or almost colourless, syrupy liquid; odourless.

Miscibility. Miscible with water and ethanol (~750 g/l) TS; slightly miscible with acetone R; practically immiscible with ether R.

Category. Solvent; humectant.

Storage. Glycerol should be kept in a tightly closed container.

Additional information. Glycerol is hygroscopic.

Requirements

Glycerol contains not less than **95.0%** and not more than the equivalent of **101.0%** of $C_3H_8O_3$, calculated with reference to the anhydrous substance.

Identity tests

A. Impregnate a piece of filter-paper with alkaline potassio-mercuric iodide TS; place it over a test-tube containing 1 ml of Glycerol with 2 g of potassium hydrogen sulfate R and heat; the paper turns black.

B. Mix 2 g with 10 ml of water and add 1 drop of phenolphthalein/ethanol TS; the solution remains colourless. Add 1 drop of methyl red/ethanol TS; the colour changes to yellow.

C. Mix 1 ml with 0.5 ml of nitric acid (~1000 g/l) TS and superimpose 0.5 ml of potassium dichromate (100 g/l) TS; a blue ring is produced at the interface of the two liquids. Allow to stand for 10 minutes; the blue colour does not diffuse into the lower layer.

Refractive index. $n_D^{20} = 1.470 - 1.475$.

Relative density. $d_{20}^{20} = 1.258 - 1.263$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 5 µg/g.

Chlorides. Use 5 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides, using 1.0 ml of hydrochloric acid CITS; the chloride content is not more than 10 µg/g.

Sulfates. Use 24 g and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 20 µg/g.

Clarity and colour of solution. Mix 25 g with sufficient water to produce 50 ml; the solution is clear. Dilute 10 ml of this solution to 25 ml with water; the solution is colourless.

Chlorinated compounds. Place about 5 g, accurately weighed, in a dry round-bottomed 100-ml flask, add 15 ml of morpholine R, and connect to a suitable reflux condenser. Reflux gently for 3 hours. Rinse the condenser with 10 ml of water, adding the washings back into the flask, and cautiously acidify with nitric acid (~1000 g/l) TS. Transfer the solution to a suitable comparison tube, add 0.5 ml of silver nitrate (0.1 mol/l) VS, dilute with water to exactly 50 ml, and mix thoroughly; the turbidity is not more pronounced than that of a solution prepared similarly but without refluxing and to which 0.2 ml of hydrochloric acid (0.02 mol/l) VS has been added (30 µg of Cl/g).

Acidity. Dilute 25 g to 50 ml with carbon-dioxide-free water R and add 0.5 ml of phenolphthalein/ethanol TS; the solution remains colourless. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS; not more than 0.2 ml is required to obtain a pink colour. (Keep the solution for "Fatty acids and esters".)

Fatty acids and esters. To the above solution, add 5 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS, boil the mixture for 5 minutes, cool, add phenolphthalein/ethanol TS, and titrate with hydrochloric acid (0.5 mol/l) VS. Repeat the procedure without the Glycerol being examined and make any necessary corrections. Not more than 1.0 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS is consumed.

Aldehydes and reducing substances. Transfer 5 ml of Glycerol to a glass-stoppered flask, mix with 10 ml of water and 1 ml of fuchsin/sulfurous acid TS. Allow the mixture to stand in the dark for 1 hour; the colour of the solution is not more intense than that of a solution of potassium permanganate (0.0002 mol/l) VS.

Sulfated ash. Not more than 0.1 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 1.5 g of Glycerol; the water content is not more than 20 mg/g.

Assay. Transfer about 0.4 g, accurately weighed, to a 600-ml beaker, dilute with 50 ml of water, add bromothymol blue/ethanol TS, and acidify with sulfuric acid (0.1 mol/l) VS to a green or greenish yellow colour. Neutralize with sodium hydroxide (0.05 mol/l) VS to a definite blue endpoint, showing no green tinge. Prepare a reagent blank containing 50 ml of water, and neutralize in the

same manner. Pipette 50 ml of sodium metaperiodate TS into each beaker, swirl gently to mix, cover with a watch-glass, and allow to stand for 30 minutes at room temperature (not exceeding 35 °C). Dilute each solution with water to about 300 ml and, using a pH-meter, titrate with sodium hydroxide (0.1 mol/l) VS to pH 8.1 ± 0.1 for Glycerol and pH 6.5 ± 0.1 for the blank. Make any necessary corrections for the blank.

Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 9.210 mg of $C_3H_8O_3$.

GLYCEROLUM 85% *m/m*
GLYCEROL 85% *m/m*

Other name. Dilute glycerol.

Description. A clear, colourless or almost colourless, syrupy liquid; odourless.

Miscibility. Miscible with water and ethanol (~750 g/l) TS; slightly miscible with acetone R; practically immiscible with ether R.

Category. Solvent; humectant.

Storage. Glycerol 85% *m/m* should be kept in a tightly closed container.

Additional information. Glycerol 85% *m/m* is hygroscopic.

Requirements

Definition. Glycerol 85% *m/m* is a mixture of glycerol and water.

Glycerol 85% *m/m* contains not less than **83.5% *m/m*** and not more than the equivalent of **88.5% *m/m*** of $C_3H_8O_3$, calculated with reference to the anhydrous substance.

Identity tests

A. Impregnate a piece of filter-paper with alkaline potassio-mercuric iodide TS; place it over a test-tube containing 1 ml of Glycerol 85% *m/m* with 2 g of potassium hydrogen sulfate R and heat; the paper turns black.

B. Mix 2 g with 10 ml of water and add 1 drop of phenolphthalein/ethanol TS; the solution remains colourless. Add 1 drop of methyl red/ethanol TS; the colour changes to yellow.

C. Mix 1 ml with 0.5 ml of nitric acid (~1000 g/l) TS and superimpose 0.5 ml of potassium dichromate (100 g/l) TS; a blue ring is produced at the interface of the two liquids. Allow to stand for 10 minutes; the blue colour does not diffuse into the lower layer.

Refractive index. $n_D^{20} = 1.449 - 1.455$.

Relative density. $d_{20}^{20} = 1.219 - 1.230$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 5 µg/g.

Chlorides. Use 5 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides, using 1.0 ml of hydrochloric acid CITS; the chloride content is not more than 10 µg/g.

Sulfates. Use 24 g and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 20 µg/g.

Clarity and colour of solution. Mix 25 g with sufficient water to produce 50 ml; the solution is clear. Dilute 10 ml of this solution to 25 ml with water; the solution is colourless.

Chlorinated compounds. Place about 5 g, accurately weighed, in a dry round-bottomed 100-ml flask, add 15 ml of morpholine R, and connect to a suitable reflux condenser. Reflux gently for 3 hours. Rinse the condenser with 10 ml of water, adding the washings back into the flask, and cautiously acidify with nitric acid (~1000 g/l) TS. Transfer the solution to a suitable comparison tube, add 0.5 ml of silver nitrate (0.1 mol/l) VS, dilute with water to exactly 50 ml, and mix thoroughly; the turbidity is not more pronounced than that of a solution prepared similarly, but without refluxing and to which 0.2 ml of hydrochloric acid (0.02 mol/l) VS has been added (30 µg of Cl/g).

Acidity. Dilute 25 g to 50.0 ml with carbon-dioxide-free water R and add 0.5 ml of phenolphthalein/ethanol TS; the solution remains colourless. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS; not more than 0.2 ml is required to obtain a pink colour. (Keep the solution for "Fatty acids and esters".)

Fatty acids and esters. To the above solution, add 5 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS, boil the mixture for 5 minutes, cool, add phenolphthalein/ethanol TS, and titrate with hydrochloric acid (0.5 mol/l) VS. Repeat the procedure without the Glycerol 85% *m/m* being examined and make any necessary corrections. Not more than 1.0 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS is consumed.

Aldehydes and reducing substances. Transfer 5 ml of Glycerol 85% *m/m* to a glass-stoppered flask, mix with 10 ml of water and 1 ml of fuchsin/sulfurous acid TS. Allow the mixture to stand in the dark for 1 hour; the colour of the solution is not more intense than that of a solution of potassium permanganate (0.0002 mol/l) VS.

Sulfated ash. Not more than 0.1 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.2 g of Glycerol 85% *m/m*; the water content is not less than 0.12 g/g and not more than 0.15 g/g.

Assay. Transfer about 0.4 g, accurately weighed, to a 600-ml beaker, dilute with 50 ml of water, add bromothymol blue/ethanol TS, and acidify with sulfuric acid (0.1 mol/l) VS to a green or greenish yellow colour. Neutralize with sodium hydroxide (0.05 mol/l) VS to a definite blue endpoint, showing no green tinge. Prepare a reagent blank containing 50 ml of water, and neutralize in the same manner. Pipette 50 ml of sodium metaperiodate TS into each beaker, swirl gently to mix, cover with a watch-glass, and allow to stand for 30 minutes at room temperature (not exceeding 35 °C). Dilute each solution with water to about 300 ml and, using a pH-meter, titrate with sodium hydroxide (0.1 mol/l) VS to pH 8.1 ± 0.1 for Glycerol 85% *m/m* and pH 6.5 ± 0.1 for the blank. Make any necessary corrections for the blank.

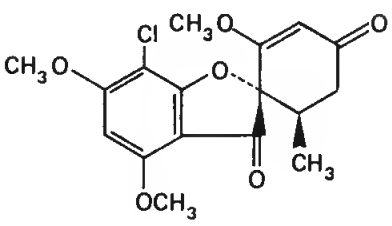
Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 9.210 mg of $C_3H_8O_3$.

GRISEOFULVINUM
GRISEOFULVIN

Molecular formula. $C_{17}H_{17}ClO_6$

Relative molecular mass. 352.8

Graphic formula.



Chemical name. 7-Chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione; (1′*S-trans*)-7-chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione; CAS Reg. No. 126-07-8.

Description. White to pale cream powder; almost odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS; freely soluble in tetrachloroethane R.

Category. Antifungal.

Storage. Griseofulvin should be kept in a well-closed container.

Additional information. The particles of Griseofulvin are generally up to 5 µm in maximum dimension, although occasionally larger particles may be present that exceed 30 µm.

Requirements

Definition. Griseofulvin contains not less than 97.0% and not more than 102.0% of $C_{17}H_{17}ClO_6$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from griseofulvin RS or with the *reference spectrum* of griseofulvin.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of ethylmethylketone R and 1 volume of xylene R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 0.50 mg of the test substance per ml and (B) 0.50 mg of griseofulvin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 5 mg in 1 ml of sulfuric acid (~1760 g/l) TS and add 5 mg of powdered potassium dichromate R; a wine-red colour is produced.

D. Melting temperature, about 220 °C.

Specific optical rotation. Use a 10 mg/ml solution in dimethylformamide R; $[\alpha]_D^{20\,°C} = +354$ to $+364°$.

Particle size. In a mortar grind 10 mg with 10 drops of hydroxyethylcellulose TS, add a further 3.50 ml of hydroxyethylcellulose TS and grind again. Transfer a drop of the suspension to a suitable counting chamber 0.10 mm deep, place a cover glass over it, and examine under a microscope 10 fields of vision of 0.04 mm^2 area each, using a magnification of 600×; not more than 30 crystals larger than 5 µm are visible in any field of vision.

Solution in dimethylformamide. A solution of 0.75 g in 10 ml of dimethylformamide R is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Matter soluble in light petroleum. Reflux 1.0 g with 40 ml of light petroleum R for 10 minutes, cool and filter. Wash the flask 3 times with 10 ml of light petroleum R, filter, evaporate the combined filtrates on a water-bath, and dry at 105 °C for 1 hour; the weight of the residue does not exceed 2.0 mg.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Acidity. Dissolve 0.25 g in 20 ml of neutralized ethanol TS and titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS, phenolphthalein/ethanol TS being used as indicator; not more than 1.0 ml is required to obtain the midpoint of the indicator (pink).

Assay. Dissolve about 0.10 g, accurately weighed, in sufficient dehydrated ethanol R to produce 200 ml and dilute 2 ml of this solution to 100 ml with dehydrated ethanol R. Determine the absorbance of this solution in a 1-cm layer at the maximum at about 291 nm and calculate the content of $C_{17}H_{17}ClO_6$, using the absorptivity value of 68.6 ($E_{1\,cm}^{1\%} = 686$).

GUMMI ARABICUM

ACACIA

Chemical name. Gum arabic; CAS Reg. No. 9000-01-5.

Description. Colourless or light yellowish brown, translucent or somewhat opaque spheroidal tears or angular fragments with numerous fissures on the

surface; very brittle; fractured surfaces are glassy and occasionally iridescent; odourless; tasteless and mucilaginous.

Solubility. Very slowly soluble in twice its mass of water, leaving only a very small residue of vegetable particles; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Viscosity-increasing agent; emulsifying agent; suspending agent; micro-encapsulating agent.

Storage. Acacia should be kept in a well-closed container, and stored in a cool place.

Additional information. Attention should be paid to the microbiological purity of Acacia since it is of natural origin. It should be enzyme-free.

Requirements

Definition. Acacia is the air-hardened, gummy exudate from the stem and branches of *Acacia Senegal* (L.) Willdenow or other species of *Acacia* of African origin; it contains mainly polymers of salts of arabic acid.

Macroscopical examination. Spheroidal, oval or reniform pieces, the diameter varying from 1–3 cm, white, yellowish white, yellow, or pale amber, sometimes with a pinkish tint, translucent or somewhat opaque, friable, frequently with a cracked surface, easily broken into transparent angular fragments with a glassy appearance and occasionally iridescent. Acacia also occurs as white to yellowish white, thin flakes, powder, or fine granules.

Microscopical examination. The flakes appear as colourless striated fragments. The powder presents colourless, bright, angular, irregular fragments with only traces of starch or vegetable tissues visible. Stratified membrane is not apparent. The granules appear as colourless, glassy, angular, irregular fragments up to 100 µm in thickness, some of which exhibit parallel linear streaks.

Identity tests (*Note*: Powder the material before performing the tests.)

A. Dissolve 1 g in 2 ml of water, add 2 ml of ethanol (~750 g/l) TS, and shake; a white, gelatinous mucilage is formed which becomes fluid on adding 10 ml of water.

B. Dissolve 0.2 g in 10 ml of water and add 4 drops of lead subacetate TS; a white, flocculent or curdy precipitate is instantaneously formed.

Starch and dextrin. Dissolve 1 g in 10 ml of water, boil and cool, then add 0.1 ml of iodine (0.05 mol/l) VS; no blue or reddish brown colour develops.

Sucrose and fructose. Dissolve 0.3 g in 5 ml of water and add 0.1 g of resorcinol R and 2 ml of hydrochloric acid (~420 g/l) TS. Heat on a water-bath for 1 minute; no yellow or pink colour develops.

Tannin. Dissolve 1 g in 10 ml of water and add 0.2 ml of ferric chloride (65 g/l) TS; a gelatinous precipitate is formed, but neither the precipitate nor the liquid shows a dark blue colour.

Solubility in water and acidity. Dissolve 1 g in 2 ml of water; the solution flows readily and is acid when tested with pH-indicator paper R.

Insoluble matter. To 5 g, add 100 ml of water and 15 ml of hydrochloric acid (~70 g/l) TS, and, while shaking frequently, boil gently for 15 minutes. Filter the hot mixture through a tared sintered-glass crucible, wash the residue with hot water, dry at 105 °C for 1 hour, and weigh; the residue weighs not more than 50 mg (1%).

Sulfated ash. Not more than 50 mg/g.

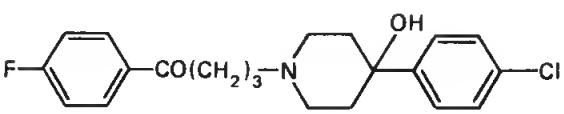
Loss on drying. Dry to constant mass at 105 °C; it loses not more than 0.15 g/g.

HALOPERIDOLUM
HALOPERIDOL

Molecular formula. $C_{21}H_{23}ClFNO_2$

Relative molecular mass. 375.9

Graphic formula.



Chemical name. 4-[4-(*p*-Chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone; 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone; CAS Reg. No. 52-86-8.

Description. A white to faintly yellowish, amorphous or microcrystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 50 parts of ethanol (~750 g/l) TS and in 200 parts of ether R.

Category. Neuroleptic.

Storage. Haloperidol should be kept in a well-closed container, protected from light.

Requirements

Definition. Haloperidol contains not less than 98.0% and not more than 101.0% of $C_{21}H_{23}ClFNO_2$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from haloperidol RS or with the *reference spectrum* of haloperidol.

B. The absorption spectrum of a 15 µg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, when observed between 230 nm and 350 nm, exhibits a maximum at about 245 nm; the absorbance of a 1-cm layer at this wavelength is between 0.49 and 0.53 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

C. Carry out the combustion as described under 2.4 Oxygen flask method, using 20 mg of the test substance and a mixture of 3 ml of sodium hydroxide (~80 g/l) TS and 2 ml of water as the absorbing liquid. When the process is complete, dilute to 10 ml with water; the resulting solution complies with the following tests:

   (*a*) Add 0.1 ml to a mixture of 0.1 ml of a freshly prepared sodium alizarinsulfonate (1 g/l) TS and 0.1 ml of zirconyl nitrate TS; the red colour of the solution changes to clear yellow.

   (*b*) Acidify 5 ml with sulfuric acid (~100 g/l) TS and boil gently for 2 minutes; the solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 147–152 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance (a precoated plate is preferable) and a mixture of 80 volumes of chloroform R, 10 volumes of glacial acetic acid R and 10 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in chloroform R containing (A) 10 mg of the test substance per ml, (B) 0.050 mg of the test substance per ml, and (C) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Not more than one of any such spots is more intense than the spot obtained with solution C.

Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 37.59 mg of $C_{21}H_{23}ClFNO_2$.

Additional requirements for Haloperidol for parenteral use

Complies with the monograph for "Parenteral preparations".

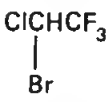
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 71.4 IU of endotoxin RS per mg.

HALOTHANUM
HALOTHANE

Molecular formula. $C_2HBrClF_3$

Relative molecular mass. 197.4

Graphic formula.



Chemical name. 2-Bromo-2-chloro-1,1,1-trifluoroethane; CAS Reg. No. 151-67-7.

Description. A colourless, mobile, heavy liquid; odour, characteristic, resembling that of chloroform.

Miscibility. Miscible with 400 parts of water; miscible with ethanol (~750 g/l) TS, ether R, and trichloroethylene R.

Category. General anaesthetic.

Storage. Halothane should be kept in a well-closed container, protected from light, and stored at a temperature not exceeding 25 °C.

Additional information. Halothane is a noninflammable liquid. Halothane contains not less than 0.08 mg/g and not more than 0.12 mg/g of thymol, as a stabilizer.

Requirements

Identity tests

To a test-tube, transfer 2 ml of *tert.*-butanol R, 0.1 ml of the test liquid, 1 ml of copper edetate TS, 0.5 ml of ammonia (~260 g/l) TS and 2 ml of hydrogen peroxide (~60 g/l) TS; this constitutes solution 1. Similarly, prepare a blank without the test liquid; this constitutes solution 2. Place both tubes in a water-bath at 50 °C for 15 minutes, cool and add 0.3 ml of glacial acetic acid R.

A. To 1 ml of each of solutions 1 and 2 add 0.5 ml of a mixture of equal volumes of sodium alizarinsulfonate (1 g/l) TS and zirconyl nitrate TS; a red colour is produced in solution 2 and a yellow colour in solution 1.

B. To 1 ml of each of solutions 1 and 2 add 1 ml of a mixture of 1.02 g of potassium hydrogen phthalate R dissolved in 30 ml of sodium hydroxide (0.1 mol/l) VS and diluted to 100 ml with water (= buffer pH 5.2). Then add (*a*) 1 ml of phenol red/ethanol TS that has been previously diluted with an equal volume of water and (*b*) 0.1 ml of tosylchloramide sodium (15 g/l) TS; a yellow colour is produced in solution 2 and a bluish red colour in solution 1.

C. To 2 ml of each of solutions 1 and 2 add 0.5 ml of sulfuric acid (~570 g/l) TS, 0.5 ml of acetone R and 0.2 ml of potassium bromate (50 g/l) TS. Shake, then place in a water-bath at 50 °C for 2 minutes. Cool, add 0.5 ml of a mixture of equal volumes of nitric acid (~1000 g/l) TS and water, and 0.1 ml of silver nitrate (40 g/l) TS; solution 2 remains clear and an opalescence is produced in solution 1, which changes to a white precipitate after a few minutes.

Mass density, ρ_{20} = 1.865–1.875 g/ml.

Free halides. Shake 10 ml with 20 ml of carbon-dioxide-free water R for 3 minutes. To 5 ml of the aqueous layer add 5 ml of water, 1 drop of nitric acid (~1000 g/l) TS, and 0.2 ml of silver nitrate (40 g/l) TS; no opalescence is produced. (Keep the remaining aqueous layer for the test of free halogens).

Free halogens. To 10 ml of the aqueous layer obtained from the test for free halides add 1 ml of potassium iodide/starch TS; no blue colour is produced.

Acidity or alkalinity. Shake 20 ml with 20 ml of carbon-dioxide-free water R for 3 minutes. To the aqueous layer add a few drops of bromocresol purple/ethanol TS; not more than 0.1 ml of sodium hydroxide (0.01 mol/l) VS or 0.6 ml of hydrochloric acid (0.01 mol/l) VS is required to obtain the midpoint of the indicator (grey).

Thymol. Use 3 dry 25-ml stoppered cylinders and place in the first one 0.5 ml of the test liquid, in the second one 0.5 ml of thymol TS2, and in the third one 0.5 ml of thymol TS3. To each of the 3 cylinders add 5 ml of carbon tetrachloride R and 5.0 ml of titanium dioxide/sulfuric acid TS. Shake the cylinders vigorously for 30 seconds and allow to stand until the layers have separated. When viewed transversally the yellowish-brown colour of the lower layer obtained in the cylinder containing the test liquid is intermediate in intensity between the colours of the corresponding layers in the other 2 cylinders (0.08–0.12 mg/g of thymol).

Related substances. Carry out the test as described under 1.14.5 Gas chromatography, using 3 solutions (1) trichlorotrifluoroethane TS serving as an internal standard, (2) the test liquid, and (3) the test liquid containing 0.05 µl of trichlorotrifluoroethane R per ml.

For the procedure use a glass column 2.75 m long and 5.0 mm in internal diameter, the first 1.8 m of which are packed with an adequate quantity of an adsorbent composed of 30 g of macrogol 400 R supported on 70 g of pink firebrick R, and the remainder with an adequate quantity of an adsorbent composed of 30 g of dinonyl phthalate R supported on 70 g of pink firebrick R. Maintain the column at 50 °C, use nitrogen R as the carrier gas and a flame ionization detector.

In the chromatogram obtained with solution 3, the peak area due to the trichlorotrifluoroethane is greater than the total area of any other peaks except that due to the halothane; in calculating the peak area due to the trichlorotrifluoroethane, allowance may be necessary for any impurities having the same retention time as revealed in the chromotogram obtained from solution 2.

HEPARINUM CALCICUM
HEPARIN CALCIUM

Chemical name. Heparin calcium; CAS Reg. No. 37270-89-6.

Description. A white or almost white powder.

Solubility. Freely soluble in water.

Category. Anticoagulant.

Storage. Heparin calcium should be kept in a tightly closed container.

Labelling. The designation Heparin calcium for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. The label should also state the name and quantity of any added substances, and the source of the material (lung or mucosal). Expiry date.

Additional information. Heparin calcium may be prepared from the lungs of oxen or the intestinal mucosae of oxen, pigs, or sheep. Attention should be paid to minimize or eliminate microbiological contamination and substances lowering blood pressure. Heparin calcium is moderately hygroscopic.

Requirements

Definition. Heparin calcium is a preparation containing the calcium salt of a sulfated glucosaminoglycan present in mammalian tissues. It has the characteristic property of delaying the clotting of fresh blood.

Heparin calcium intended for the manufacture of a parenteral dosage form contains not less than **150 IU per mg**, and Heparin calcium not intended for use in the manufacture of a parenteral dosage form contains not less than **120 IU per mg**, both calculated with reference to the dried substance.

Identity tests

A. Delays the clotting of fresh blood.

B. Specific optical rotation, use a 40 mg/ml solution; $[\alpha]_D^{20\,°C}$ = not less than +35 °.

C. A 20 mg/ml solution yields the reactions described under 2.1 General identification tests as characteristic of calcium.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 30 µg/g.

Calcium. Proceed with about 0.2 g, accurately weighed, as described under 2.5 Complexometric titrations for calcium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.004 mg of Ca; 95–115 mg/g, calculated with reference to the dried substance. (As an alternative, determine the content of calcium by atomic absorption spectrophotometry under 1.8 Atomic spectrometry: emission and absorption.)

Nitrogen. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.1 g, accurately weighed, and 5 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of nitrogen; not more than 25 mg/g, with reference to the dried substance.

Protein and nucleotidic impurities. Measure the absorbance of a 1-cm layer of a 4 mg/ml solution at a wavelength of 260 nm and 280 nm; at 260 nm not greater than 0.20 and at 280 nm not greater than 0.15.

Clarity and colour of solution. A solution containing 5000 IU per ml is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Use 0.2 g; 0.32–0.40 g/g, with reference to the dried substance.

Loss on drying. Dry to constant mass at 60 °C under reduced pressure (not exceeding 0.5 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 0.080 g/g.

pH value. pH of 10 mg/ml solution in carbon-dioxide-free water R, 5.5–8.0.

Assay. The anticoagulant activity of heparin is determined *in vitro* using a biological assay to compare its ability to delay the clotting of recalcified citrated sheep plasma with that of the reference substance. The following method is suitable for carrying out the assay *(other methods may also be applicable)*.

The onset of clotting is determined either as a change in optical density (by direct visual inspection, preferably using indirect illumination against a matt black background, or by spectrophotometry, recording at a wavelength of approximately 600 nm) or as a change in fluidity (by visual detection while manually tilting the tube or by mechanical recording while stirring, taking care to cause the minimum disturbance of the solution during the initial phase of clotting). Use appropriate tubes according to the chosen technique.

Prepare a solution of Heparin calcium and a solution of heparin RS in sodium chloride (9 g/l) TS, each containing an accurately known number of IU of heparin per ml. Using sodium chloride (9 g/l) TS prepare from each solution a series of dilutions in geometric progression such that the clotting time obtained with the lowest concentration is not less than 1.5 times the blank recalcification time, and the clotting time obtained with the highest concentration is such as to give a satisfactory log dose-response curve, as determined in a preliminary test.

Place in an ice-bath 12 labelled tubes for each dilution: T_1, T_2, T_3, etc. for Heparin calcium and S_1, S_2, S_3, etc. for heparin RS. To each tube add 1.0 ml of thawed plasma substrate R and 1.0 ml of the appropriate dilution, either from Heparin calcium or heparin RS, mixing each tube carefully, and not allowing bubbles to form. (The detection technique employed may require the addition of different volumes of plasma substrate, consequently the appropriate adjustment of all tubes would be needed.) Transfer all the tubes to a water-bath at 37 °C, and allow to equilibrate for about 15 minutes. Add to each tube, mixing after each addition, 1 ml of a dilution of cephalin TS and 1 ml of kaolin suspension TS freshly prepared just before use. (A suitable dilution of cephalin TS is one that, under the conditions of the assay, gives a blank recalcification time of not more than 60 seconds.) After exactly 2 minutes, add 1.0 ml of calcium chloride (3.7 g/l) TS. Record in seconds the interval between this addition and the onset of clotting, determined according to the chosen technique. Similarly determine the blank recalcification time at the beginning and at the end of the procedure, using 1.0 ml of sodium chloride (9 g/l) TS in place of one of the heparin dilutions; the two values for the blank should not differ significantly. Repeat the procedure using fresh dilutions of the initial solutions and carrying out the incubation in the reverse order (first tubes S, then tubes T).

Transform the clotting times to logarithms using the mean values for the duplicate tubes and calculate the results by standard statistical methods.

Carry out not fewer than 3 independent assays. For each assay prepare fresh solutions of Heparin calcium and heparin RS, and use a different, freshlythawed portion of the stored plasma substrate R.

Calculate the potency of Heparin calcium by combining the results of the assays by standard statistical methods. If the variance is significant ($P = 0.01$), due to differences between assays, it is possible to obtain a combined estimate by calculating the non-weighted mean of potency estimates.

The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 80% and not more than 125% of the stated potency.

Additional requirements for Heparin calcium for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; Heparin calcium intended for the manufacture of a parenteral dosage form, without further appropriate procedure for the removal of bacterial endotoxins, contains not more than 0.01 IU of endotoxin RS per IU of heparin activity. The addition of divalent cations may be necessary in order to fulfil the validation criteria.

HEPARINUM NATRICUM
HEPARIN SODIUM

Chemical name. Heparin sodium; CAS Reg. No. 9041-08-1.

Description. A white or almost white powder.

Solubility. Freely soluble in water.

Category. Anticoagulant.

Storage. Heparin sodium should be kept in a tightly closed container.

Labelling. The designation Heparin sodium for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. The label should also state the name and quantity of any added substances, and the source of the material (lung or mucosal). Expiry date.

Additional information. Heparin sodium may be prepared from the lungs of oxen or the intestinal mucosae of oxen, pigs, or sheep. Attention should be paid to minimize or eliminate microbiological contamination and substances lowering blood pressure. Heparin sodium is moderately hygroscopic.

Requirements

Definition. Heparin sodium is a preparation containing the sodium salt of a sulfated glucosaminoglycan present in mammalian tissues. It has the characteristic property of delaying the clotting of fresh blood.

Heparin sodium intended for the manufacture of a parenteral dosage form contains not less than **150 IU per mg**, and Heparin sodium not intended for use

in the manufacture of a parenteral dosage form contains not less than **120 IU per mg**, both calculated with reference to the dried substance.

Identity tests

A. Delays the clotting of fresh blood.

B. Specific optical rotation, use a 40 mg/ml solution; $[\alpha]_D^{20\,°C}$ is not less than +35°.

C. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 30 µg/g.

Sodium. Determine by atomic absorption spectrophotometry under 1.8 Atomic spectrometry: emission and absorption at a wavelength of 330.3 nm, using a sodium hollow cathode lamp and a flame of suitable composition (e.g. 11 litres of air and 2 litres of acetylene per minute). Prepare a solution of 5 mg in 10 ml of hydrochloric acid (0.1 mol/l) VS containing 1.27 mg/ml of caesium chloride R. As a reference solution use sodium standard (200 µg of Na per ml) TS and use dilutions containing 25, 50, and 75 µg of Na per ml in the same mixture of caesium chloride and hydrochloric acid as prepared above; 95–125 mg of Na per g, calculated with reference to the dried substance.

Nitrogen. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.1 g, accurately weighed, and 5 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of nitrogen; not more than 25 mg/g, with reference to the dried substance.

Protein and nucleotidic impurities. Measure the absorbance of a 1-cm layer of a 4 mg/ml solution at a wavelength of 260 nm and 280 nm; at 260 nm not greater than 0.20 and at 280 nm not greater than 0.15.

Clarity and colour of solution. A solution containing 5000 IU per ml is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Use 0.2 g; 0.30–0.43 g/g, with reference to the dried substance.

Loss on drying. Dry to constant mass at 60 °C under reduced pressure (not exceeding 0.5 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 0.080 g/g.

pH value. pH of 10 mg/ml solution in carbon-dioxide-free water R, 5.5–8.0.

Assay. The anticoagulant activity of heparin is determined *in vitro* using a biological assay to compare its ability to delay the clotting of recalcified citrated sheep plasma with that of the reference substance. The following method is suitable for carrying out the assay *(other methods may also be applicable).*

The onset of clotting is determined either as a change in optical density (by direct visual inspection, preferably using indirect illumination against a matt black background, or by spectrophotometry, recording at a wavelength of approximately 600 nm) or as a change in fluidity (by visual detection while manually tilting the tube or by mechanical recording while stirring, taking care to cause the minimum disturbance of the solution during the initial phase of clotting). Use appropriate tubes according to the chosen technique.

Prepare a solution of Heparin sodium and a solution of heparin RS in sodium chloride (9 g/l) TS, each containing an accurately known number of IU of heparin per ml. Using sodium chloride (9 g/l) TS prepare from each solution a series of dilutions in geometric progression such that the clotting time obtained with the lowest concentration is not less than 1.5 times the blank recalcification time, and the clotting time obtained with the highest concentration is such as to give a satisfactory log dose-response curve, as determined in a preliminary test.

Place in an ice-bath 12 labelled tubes for each dilution: T_1, T_2, T_3, etc. for Heparin sodium and S_1, S_2, S_3, etc. for heparin RS. To each tube add 1.0 ml of thawed plasma substrate R and 1.0 ml of the appropriate dilution, either from Heparin sodium or heparin RS, mixing each tube carefully, and not allowing bubbles to be formed. (The detection technique employed may require the addition of different volumes of plasma substrate, consequently the appropriate adjustment of all tubes would be needed.) Transfer all the tubes to a water-bath at 37 °C, and allow to equilibrate for about 15 minutes. Add to each tube, mixing after each addition, 1 ml of a dilution of cephalin TS and 1 ml of kaolin suspension TS freshly prepared just before use. (A suitable dilution of cephalin TS is one that, under the conditions of the assay, gives a blank recalcification time of not more than 60 seconds.) After exactly 2 minutes, add 1.0 ml of calcium chloride (3.7 g/l) TS. Record in seconds the interval between this addition and the onset of clotting, determined according to the chosen technique. Similarly determine the blank recalcification time at the beginning and at the end of the procedure, using 1.0 ml of sodium chloride (9 g/l) TS in place of one of the heparin dilutions; the two values for the blank should not differ significantly. Repeat the procedure using fresh dilutions of the initial solutions and carrying out the incubation in the reverse order (first tubes S, then tubes T).

Transform the clotting times to logarithms using the mean values for the duplicate tubes and calculate the results by standard statistical methods.

Carry out not fewer than 3 independent assays. For each assay prepare fresh solutions of Heparin sodium and heparin RS, and use a different, freshlythawed portion of the stored plasma substrate R.

Calculate the potency of Heparin sodium by combining the results of the assays by standard statistical methods. If the variance is significant ($P = 0.01$), due to differences between assays, it is possible to obtain a combined estimate by calculating the non-weighted mean of potency estimates.

The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 80% and not more than 125% of the stated potency.

Additional requirements for Heparin sodium for parenteral use

Complies with the monograph for "Parenteral preparations".

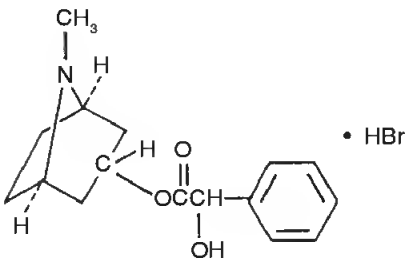
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; Heparin sodium intended for the manufacture of a parenteral dosage form, without further appropriate procedure for the removal of bacterial endotoxins, contains not more than 0.01 IU of endotoxin RS per IU of heparin activity. The addition of divalent cations may be necessary in order to fulfil the validation criteria.

HOMATROPINI HYDROBROMIDUM
HOMATROPINE HYDROBROMIDE

Molecular formula. $C_{16}H_{21}NO_3,HBr$

Relative molecular mass. 356.3

Graphic formula.



Chemical name. 1α*H*,5α*H*-Tropan-3α-ol mandelate (ester) hydrobromide; (±)-*endo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl α-hydroxybenzeneacetate hydrobromide; CAS Reg. No. 51-56-9.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Mydriatic.

Storage. Homatropine hydrobromide should be kept in a tightly closed container, protected from light.

Requirements

Definition. Homatropine hydrobromide contains not less than 98.5% and not more than 101.0% of $C_{16}H_{21}NO_3$,HBr, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add ammonia (~100 g/l) TS to render the solution slightly alkaline, and shake with 5 ml of chloroform R. Evaporate the chloroform layer to dryness on a water-bath and add 1.5 ml of mercuric chloride/ethanol TS to the residue; a yellow colour is produced, which turns red on heating.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of bromides.

C. Melting temperature, about 215 °C with decomposition.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

pH value. pH of a 20 mg/ml solution, 5.5–7.0.

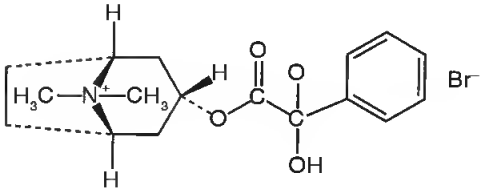
Foreign alkaloids. Dissolve 10 mg in 2 ml of water and add 0.25 ml of tannic acid (50 g/l) TS; no precipitate is produced.

Related alkaloids. Dissolve 5 mg in 0.25 ml of fuming nitric acid R and evaporate to dryness on a water-bath. Allow to cool, add 0.1 ml of acetone R and 0.1 ml of a mixture of 1 volume of potassium hydroxide/ethanol (0.5 mol/l) VS

and 4 volumes of aldehyde-free ethanol (~750 g/l) TS; no violet or reddish violet colour is produced.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 35.63 mg of $C_{16}H_{21}NO_3$,HBr.

HOMATROPINI METHYLBROMIDUM
HOMATROPINE METHYLBROMIDE



$C_{17}H_{24}BrNO_3$

Relative molecular mass. 370.3

Chemical name. 3α-Hydroxy-8-methyl-1α*H*,5α*H*-tropanium bromide (±)-mandelate;(±)-*endo*-3-[(hydroxyphenylacetyl)oxy]-8,8-dimethyl-8-azonia-bicyclo-[3.2.1]octane bromide; CAS Reg. No. 80-49-9.

Description. A white, crystalline powder; odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R and acetone R.

Category. Mydriatic.

Storage. Homatropine methylbromide should be kept in a tightly closed container, protected from light.

Additional information. Homatropine methylbromide darkens on exposure to light.

Requirements

Homatropine methylbromide contains not less than **98.5%** and not more than the equivalent of **101.0%** of $C_{17}H_{24}BrNO_3$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add ammonia (~100 g/l) TS to render the solution slightly alkaline, and shake with 5 ml of chloroform R. Evaporate the chloroform layer to dryness on a water-bath and add 1.5 ml of mercuric chloride/ethanol TS to the residue; no yellow or red colour is produced (distinction from homatropine, atropine, and other solanaceous alkaloids).

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of bromides.

C. Melting temperature, about 190 °C.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 6 volumes of 1-propanol R, 3 volumes of water, 2 volumes of methanol R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in a mixture of 9 volumes of methanol R and 1 volume of water containing (A) 40 mg of Homatropine methylbromide per ml and (B) 0.4 mg of Homatropine methylbromide per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it first with potassium iodobismuthate TS2 and then with sodium nitrite (50 g/l) TS. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.7 g, accurately weighed, in 50 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A.

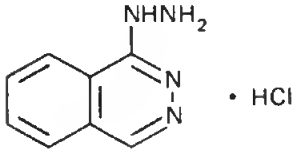
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 37.03 mg of $C_{17}H_{24}BrNO_3$.

HYDRALAZINI HYDROCHLORIDUM
HYDRALAZINE HYDROCHLORIDE

Molecular formula. $C_8H_8N_4,HCl$

Relative molecular mass. 196.6

Graphic formula.



Chemical name. 1-Hydrazinophthalazine monohydrochloride; 1(2*H*)-phthalazinone hydrazone monohydrochloride; CAS Reg. No. 304-20-1.

Other name. Apressinum.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 25 parts of water; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Antihypertensive drug.

Storage. Hydralazine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Hydralazine hydrochloride melts at about 275 °C with decomposition. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Hydralazine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_8H_8N_4,HCl$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution, when observed between 220 nm and 350 nm, exhibits maxima at 240 nm, 260 nm, 303 nm, and

315 nm; the absorbances of a 1-cm layer at these wavelenghts are about 0.58, 0.54, 0.27, and 0.21, respectively.

B. Dissolve 0.5 g in a mixture of 100 ml of water and 8 ml of hydrochloric acid (~70 g/l) TS, add 20 ml of sodium nitrite (10 g/l) TS, allow to stand for 10 minutes and filter. Wash the residue with water and dry at 105 °C; melting temperature, about 210 °C.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Water-insoluble substances. Transfer 2.0 g to a 250-ml conical flask, add 100 ml of water, and shake by mechanical means for 30 minutes. Filter the solution through a tared sintered glass crucible, rinse the flask, and wash any undissolved residue into the crucible. Wash the residue with three 10-ml portions of water, dry at 105 °C for 3 hours, cool and weigh; the residue weighs not more than 10 mg (5 mg/g).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 8 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance. For the mobile phase, shake a mixture of 2 volumes of ethyl acetate R, 2 volumes of ammonia (~260 g/l) TS and 8 volumes of hexane R, allow to separate and use the upper layer. For the preparation of the test solution dissolve 0.10 g of the test substance in a mixture of 100 volumes of methanol R and 1 volume of hydrochloric acid (~420 g/l) TS, and dilute to 20 ml with the same solvent mixture; to 2.0 ml of this solution add 1.0 ml of salicylaldehyde TS, centrifuge and decant the supernatant liquid (solution A). For the reference solution, dissolve 25.0 mg of hydrazine sulfate R in 10 ml of water and dilute to 100 ml using a mixture of 1 volume of hydrochloric acid (~420 g/l) TS and 100 volumes of methanol R; dilute 1.0 ml to 100 ml with the same solvent mixture. To 2.0 ml of this solution add 1.0 ml of salicylaldehyde TS, centrifuge and decant the supernatant liquid (solution B). Apply separately to the plate 40 µl of each of solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air and spray with 4-dimethylaminobenzaldehyde TS6. Examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.15 g, accurately weighed, in 25 ml of water, add 25 ml of hydrochloric acid (~420 g/l) TS, cool to room temperature, add 5 ml of chloroform R, and titrate with potassium iodate (0.05 mol/l) VS, shaking continu-

ously, until the purple colour of iodine in the chloroform layer disappears. The endpoint is reached when the chloroform layer remains colourless for at least 5 minutes. Each ml of potassium iodate (0.05 mol/l) VS is equivalent to 9.832 mg of $C_8H_8N_4,HCl$.

HYDRARGYRI OXYCYANIDUM
MERCURIC OXYCYANIDE

Chemical name. CAS Reg. No. 73360-53-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 20 parts of water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antiseptic (for topical use).

Storage. Mercuric oxycyanide should be kept in a tightly closed container, protected from light.

Additional information. Mercuric oxycyanide is slowly discoloured by light. It might explode on triturating or mixing with other substances. The solution should not be heated on an open flame.

Requirements

Definition. Mercuric oxycyanide is a mixture of approximately 1 part of $Hg(CN)_2,HgO$ and 2 parts of $Hg(CN)_2$.

Mercuric oxycyanide contains not less than 14.5% and not more than 17.2% of HgO, and not less than 82.5% and not more than 85.5% of $Hg(CN)_2$.

Identity tests

A. Immerse a small piece of copper plate in a 0.05 g/ml solution for some minutes, remove, wash with water, and rub the plate with paper; a bright silvery surface is produced.

B. To a 0.05 g/ml solution add potassium iodide (80 g/l) TS; a yellow solution is produced which on the addition of ammonia (~100 g/l) TS gives a reddish brown precipitate.

C. To 1 ml of a 0.05 g/ml solution add 0.05 g of ferrous sulfate R and sufficient sodium hydroxide (~80 g/l) TS to precipitate the iron as hydroxide. Boil the

mixture and acidify with hydrochloric acid (~70 g/l) TS; a blue colour or precipitate is produced.

Chlorides. Dissolve 1.75 g in 20 ml of water, add 10 ml of sodium hydroxide (~80 g/l) TS and 25 ml of formaldehyde TS, and boil gently for 10 minutes. Cool, filter, wash the precipitate with water, combine the filtrate and washings, neutralize with nitric acid (~130 g/l) TS and add sufficient water to produce 100 ml. Proceed as described under 2.2.1 Limit test for chlorides, using 40 ml of this solution and 6 ml of nitric acid (~130 g/l) TS. To prepare the standard opalescence mix 10 ml of sodium hydroxide (~80 g/l) TS with 12.25 ml of hydrochloric acid ClTS, and boil the mixture gently for 10 minutes. Cool, filter, wash the filter with water, combine the filtrate and washings, neutralize with nitric acid (~130 g/l) TS, and add sufficient water to produce 100 ml. Proceed as described under 2.2.1 Limit test for chlorides, using 40 ml of this solution and 6 ml of nitric acid (~130 g/l) TS; the chloride content is not more than 0.35 mg/g.

Clarity of solution. A solution of 0.050 g in 10 ml of water is clear.

Sulfated ash. Carry out the ignition under a hood; not more than 2.5 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 7.4–8.0.

Assay

For mercuric oxide. Dissolve about 0.5 g, accurately weighed, in 50 ml of water, add 1 g of sodium chloride R and titrate with hydrochloric acid (0.1 mol/l) VS using methyl orange/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 10.83 mg of HgO. (Keep the solution for the assay for mercuric cyanide).

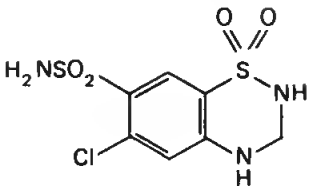
For mercuric cyanide. Use the solution obtained from the assay for mercuric oxide, add 3 g of potassium iodide R, and continue to titrate with hydrochloric acid (0.1 mol/l) VS. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 12.63 mg of $Hg(CN)_2$.

HYDROCHLOROTHIAZIDUM
HYDROCHLOROTHIAZIDE

Molecular formula. $C_7H_8ClN_3O_4S_2$

Relative molecular mass. 297.7

Graphic formula.



Chemical name. 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; CAS Reg. No. 58-93-5.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; practically insoluble in ether R; soluble in 200 parts of ethanol (~750 g/l) TS and in 20 parts of acetone R.

Category. Diuretic.

Storage. Hydrochlorothiazide should be kept in a well-closed container.

Requirements

Definition. Hydrochlorothiazide contains not less than 98.0% and not more than 102.0% of $C_7H_8ClN_3O_4S_2$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from hydrochlorothiazide RS or with the *reference spectrum* of hydrochlorothiazide.

B. Mix 10 mg of the test substance and 10 mg of disodium chromotropate R, add 1 ml of water and, cautiously, 5 ml of sulfuric acid (~1760 g/l) TS; a purple colour is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Free chlorides. For the preparation of the test solution shake 0.3 g with 20 ml of water and 10 ml of nitric acid (~130 g/l) TS for 5 minutes, and filter. Proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.8 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Diazotizable substances. For the test solution place about 0.01 g, accurately weighed, in a 50-ml volumetric flask, and dissolve in 10 ml of methanol R. Dilute to volume with water and mix.

For the reference solution weigh 5.0 mg of 4-amino-6-chloro-1,3-benzenedisulfonamide R, transfer to a 10-ml volumetric flask, and dissolve in 1 ml of methanol R. Dilute to volume with water and mix. Dilute 4 ml of this solution with sufficient water to produce 100 ml (=20 µg/ml).

Transfer 5 ml of the test solution and of the reference solution to separate 50-ml volumetric flasks, and 5 ml of water to a third 50-ml volumetric flask to serve as a blank. To each flask add 1 ml of freshly prepared sodium nitrite (10 g/l) TS and 5 ml of hydrochloric acid (~70 g/l) TS, and allow to stand for 5 minutes. Add 2 ml of ammonium sulfamate (25 g/l) TS, allow to stand for 5 minutes with frequent swirling, then add 2 ml of freshly prepared disodium chromotropate (10 g/l) TS and 10 ml of sodium acetate (150 g/l) TS. Dilute with water to volume and mix. Measure the absorbance at the maximum at about 500 nm, against the blank. The absorbance of the test solution does not exceed that of the reference solution (10 mg/g).

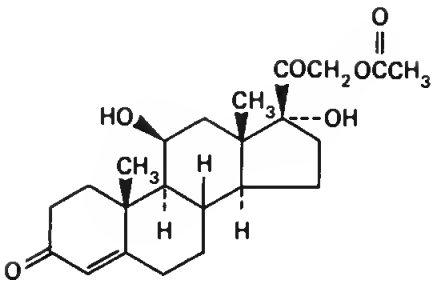
Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of pyridine R, add 5 drops of azo violet TS and titrate quickly with sodium methoxide (0.1 mol/l) VS to a deep blue endpoint, as described under 2.6 Non-aqueous titration. Method B. Each ml of sodium methoxide (0.1 mol/l) VS is equivalent to 14.89 mg of $C_7H_8ClN_3O_4S_2$.

HYDROCORTISONI ACETAS
HYDROCORTISONE ACETATE

Molecular formula. $C_{23}H_{32}O_6$

Relative molecular mass. 404.5

Graphic formula.



Chemical name. 21-(Acetyloxy)-11β,17-dihydroxypregn-4-ene-3,20-dione; 11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate; CAS Reg. No. 50-03-3.

Other name. Cortisol acetate.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Adrenocortical steroid.

Storage. Hydrocortisone acetate should be kept in a well-closed container, protected from light.

Additional information. Hydrocortisone acetate melts at about 220 °C with decomposition.

Requirements

Definition. Hydrocortisone acetate contains not less than 97.0% and not more than 102.0% of $C_{23}H_{32}O_6$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from hydrocortisone acetate RS or with the *reference spectrum* of hydrocortisone acetate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent

has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours and carry out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 75 volumes of toluene R and 25 volumes of chloroform R. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of hydrocortisone acetate RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with sulfuric acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +157$ to +168 °.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R2 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R and 0.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated; then heat at 105 °C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

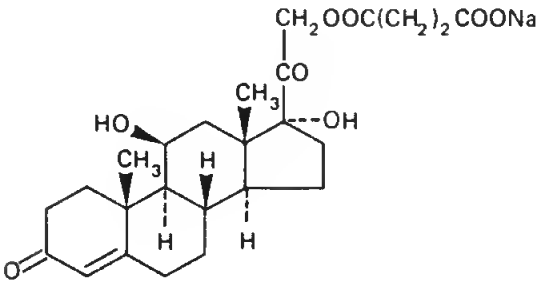
Assay. Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 242 nm. Calculate the amount of $C_{23}H_{32}O_6$ in the substance being tested by comparison with hydrocortisone acetate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.40 ± 0.02 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

HYDROCORTISONI NATRII SUCCINAS
HYDROCORTISONE SODIUM SUCCINATE

Molecular formula. $C_{25}H_{33}NaO_8$

Relative molecular mass. 484.5

Graphic formula.



Chemical name. Cortisol 21-(sodium succinate); 21-(3-carboxy-1-oxopropoxy)-11β,17-dihydroxypregn-4-ene-3,20-dione monosodium salt; CAS Reg. No. 125-04-2.

Description. A white or almost white, crystalline powder or amorphous solid; odourless.

Solubility. Freely soluble in water; soluble in 34 parts of ethanol (~750 g/l) TS and in 200 parts of dehydrated ethanol R; practically insoluble in ether R.

Category. Adrenal hormone.

Storage. Hydrocortisone sodium succinate should be kept in a tightly closed container, protected from light.

Additional information. Hydrocortisone sodium succinate is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Hydrocortisone sodium succinate contains not less than 97.0% and not more than 103.0% of $C_{25}H_{33}NaO_8$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from hydrocortisone sodium succinate RS or with the *reference spectrum* of hydrocortisone sodium succinate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a freshly prepared mixture of 3 volumes of 1-butanol R, 1 volume of acetic anhydride R, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of hydrocortisone sodium succinate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with a mixture of 10 ml of sulfuric acid (~1760 g/l) TS and 90 ml of ethanol (~750 g/l) TS, heat it at 120 °C for 10 minutes, allow it to cool, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. When tested for sodium as described under 2.1 General identification tests, it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Specific optical rotation. Use a 10 mg/ml solution in ethanol (~750 g/l) TS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +140\,°$ to $+150\,°$.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 30 mg/g.

Related steroids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of equal volumes of chloroform R and methanol R containing (A) 15 mg of the test substance per ml and (B) 0.15 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, then heat it at 105 °C for 10 minutes; allow it to cool, spray it with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Sodium. Dissolve with gentle heating about 1 g, accurately weighed, in 75 ml of glacial acetic acid R. Add 20 ml of dioxan R, 0.15 ml of crystal violet/acetic

acid TS, and titrate with perchloric acid (0.1 mol/l) VS. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 2.299 mg of Na; the content is not less than 46.0 mg and not more than 48.4 mg of Na per g, calculated with reference to the dried substance.

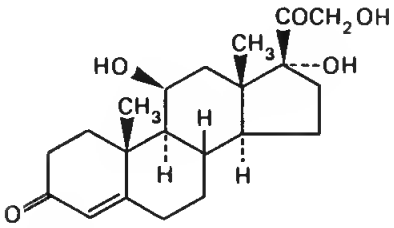
Assay. Dissolve about 20 mg, accurately weighed, in sufficient water to produce 100 ml; dilute 5 ml to 100 ml with water. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 248 nm. Calculate the amount of $C_{25}H_{33}NaO_8$ in the substance being tested by comparison with hydrocortisone sodium succinate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.34 ± 0.02.

HYDROCORTISONUM
HYDROCORTISONE

Molecular formula. $C_{21}H_{30}O_5$

Relative molecular mass. 362.5

Graphic formula.



Chemical name. 11β,17,21-Trihydroxypregn-4-ene-3,20-dione; CAS Reg. No. 50-23-7.

Other name. Cortisol.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Very slightly soluble in water and ether R; sparingly soluble in ethanol (~750 g/l) TS and acetone R.

Category. Adrenocortical steroid.

Storage. Hydrocortisone should be kept in a well-closed container, protected from light.

Additional information. Hydrocortisone melts at about 214 °C with decomposition.

Requirements

Definition. Hydrocortisone contains not less than 97.0% and not more than 102.0% of $C_{21}H_{30}O_5$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from hydrocortisone RS or with the *reference spectrum* of hydrocortisone.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml, and (B) 2.5 mg of hydrocortisone RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with sulfuric acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +150$ to +156 °.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 105 °C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 242 nm. Calculate the amount of $C_{21}H_{30}O_5$ in the substance being tested by comparison with hydrocortisone RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.44 ± 0.02 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

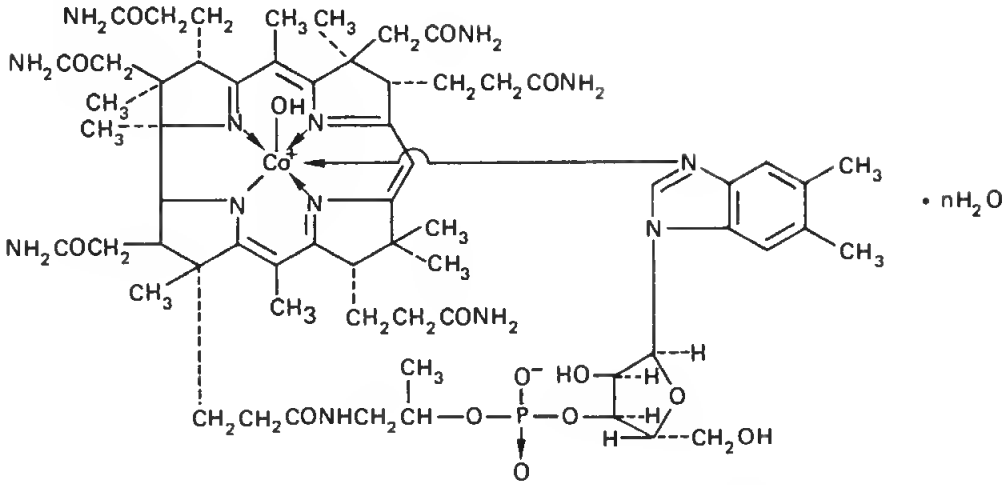
HYDROXOCOBALAMINI CHLORIDUM
HYDROXOCOBALAMIN CHLORIDE

HYDROXOCOBALAMINI SULFAS
HYDROXOCOBALAMIN SULFATE

Molecular formula. $C_{62}H_{90}ClCoN_{13}O_{15}P$; $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$.

Relative molecular mass. 1383 (hydroxocobalamin chloride); 2791 (hydroxocobalamin sulfate).

Graphic formula for the base.



Chemical name. Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole monohydrochloride; cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole monohydrochloride; *Co*α-[α-(5,6-dimethylbenzimidazolyl)]-*Co*β-hydroxocobamide chloride; CAS Reg. No. 59461-30-2.

Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole sulfate (salt) (2:1); cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole sulfate (salt) (2:1); 2(*Co*α-[α-(5,6-dimethylbenzimidazolyl)]-*Co*β-hydroxocobamide) sulfate (salt) (2:1).

Description. Dark red crystals or a red, crystalline powder; odourless.

Solubility. Soluble in water.

Category. Antianaemia drug.

Storage. Hydroxocobalamin chloride or sulfate should be kept in a tightly closed container, protected from light, and stored in a cool place.

Labelling. The designation on the container should state whether the substance is the chloride or the sulfate salt.

Additional information. Even in the absence of light, Hydroxocobalamin chloride and Hydroxocobalamin sulfate are gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Hydroxocobalamin chloride contains not less than 96.0% and not more than 102.0% of $C_{62}H_{90}ClCoN_{13}O_{15}P$, calculated with reference to the dried substance; Hydroxocobalamin sulfate contains not less than 96.0% and not more than 102.0% of $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 40 µg/ml solution in pH 4.5 acetate buffer TS, when observed between 230 nm and 550 nm, exhibits 3 maxima at about 274 nm, 351 nm, and 525 nm; the ratio of the absorbance of a 1-cm layer at 525 nm to that at 351 nm is about 0.34, and the ratio of the absorbance at 274 nm to that at 351 nm is about 0.80.

B. Heat cautiously about 2 mg in a porcelain crucible with a few drops of sulfuric acid (~1760 g/l) TS until a faintly bluish residue is produced. Cool, add 0.05 ml of water and then a few drops of a saturated solution of ammonium thiocyanate R; a blue-green colour is produced.

C. Place about 2 mg in a 100-ml glass-stoppered flask, dissolve in 2 ml of water and add 5 ml of phosphoric acid (~1440 g/l) TS. Insert in the flask a flat-bottomed glass tube 1 cm in diameter and 2 cm long, containing 1 ml of lithium carbonate/trinitrophenol TS. Close the flask and expose it for 4 hours to a bright light; the colour of the reagent in the glass tube remains unchanged (distinction from cyanocobalamin).

D. For the chloride salt prepare a 20 mg/ml solution. It yields reaction B described under 2.1 General identification tests as characteristic of chlorides. For the sulfate salt prepare a 20 mg/ml solution. It yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Loss on drying. Dry at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 2 hours; the chloride salt loses between 80 mg/g and 120 mg/g and the sulfate salt between 80 mg/g and 160 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 8.0–10.0.

Other cobalamins. Carry out the test as described under 1.14.3 Column chromatography shaking 20 g of diethylaminoethylcellulose R with 200 ml of sodium hydroxide (0.5 mol/l) VS, dilute with water to obtain a homogeneous suspension,

allow to settle, and discard the supernatant liquid. Using a suitable filter, wash with water until the washings are free from alkali, then transfer the adsorbent to a tube, length 22 cm, diameter 1.2 cm, and provided with a stopcock. Allow to settle and tap the tube until the height of the adsorbent is about 14 cm. Wash with water until the pH of the eluate is the same as that of the water.

Similarly prepare a second column, slurrying carboxymethylcellulose R with hydrochloric acid (0.5 mol/l) VS, dilute with water, allow to settle, and discard the supernatant liquid. Using a suitable filter, wash with water until the washings are free from acid, then transfer the adsorbent to a tube, length 22 cm, diameter 1.2 cm, and provided with a stopcock. Allow to settle and tap the tube until the height of the adsorbent is about 10 cm. Wash with water until the pH of the eluate is the same as that of the water.

Cover each column with a plug of glass wool, and allow to drain until only a small amount of water remains above the adsorbents.

Place the column of diethylaminoethylcellulose above the other column so that the effluent runs into the carboxymethylcellulose.

Weigh accurately about 0.05 g of the substance to be examined, dissolve it in 20 ml of water, and acidify with sufficient hydrochloric acid (~70 g/l) TS to obtain a pH of 4.0. Introduce this solution to the diethylaminoethylcellulose column and allow it to run through both columns, rejecting the first colourless eluate. Elute with water the pH of which has previously been adjusted to 4.0 with hydrochloric acid (~70 g/l) TS. Collect the coloured eluate into a 50-ml volumetric flask and adjust to volume with water. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 361 nm, and calculate the content of other cobalamins in mg/g, using the absorptivity value of 20.7 ($A^{1\%}_{1\,cm}$ = 207); not more than 30 mg/g.

Acidic impurities. Elute the diethylaminoethylcellulose column from the above test for other cobalamins with sodium chloride (10 g/l) TS, collecting 50 ml of eluate. Measure the absorbance of this solution in a 1-cm layer at the maximum between 351 nm and 361 nm, and calculate the content of acidic impurities in mg/g, using the absorptivity value of 19.0 ($A^{1\%}_{1\,cm}$ = 190); not more than 30 mg/g.

Assay

- The solutions must be protected from light throughout the assay.

Dissolve about 20 mg, accurately weighed, in sufficient acetate buffer, pH 4.5, TS to produce 500 ml. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 351 nm and calculate the content of $C_{62}H_{90}ClCoN_{13}O_{15}P$ or $C_{124}H_{180}Co_2N_{26}O_{34}P_2S$, using the absorptivity values of 19.0 or 18.8, respectively ($A^{1\%}_{1\,cm}$ = 190 or 188, respectively).

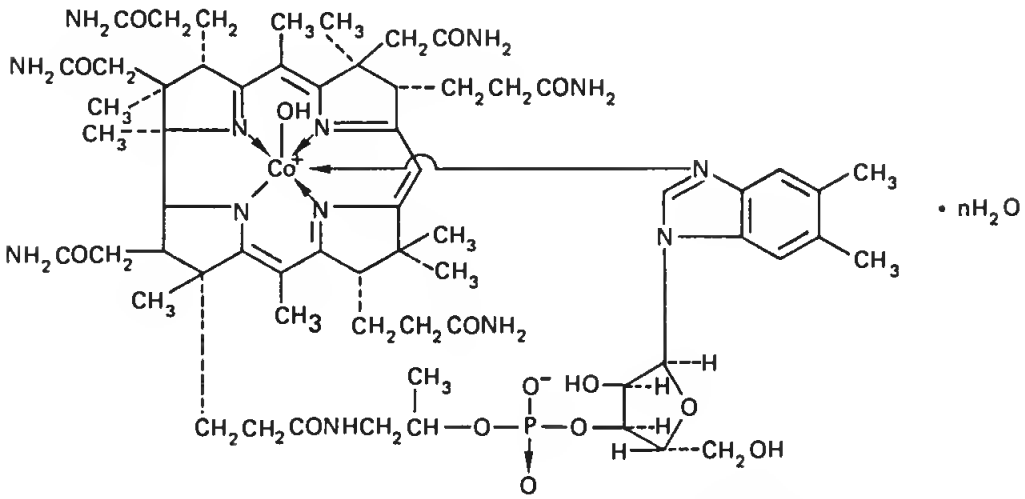
HYDROXOCOBALAMINUM
HYDROXOCOBALAMIN

Hydroxocobalamin anhydrous
Hydroxocobalamin hydrate

Molecular formula. $C_{62}H_{89}CoN_{13}O_{15}P$ (anhydrous); $C_{62}H_{89}CoN_{13}O_{15}P,H_2O$ (monohydrate).

Relative molecular mass. 1346 (anhydrous); 1365 (monohydrate).

Graphic formula.



Chemical name. Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole; cobinamide dihydrogen phosphate (ester)-mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole; *Co*α-[α-(5,6-dimethylbenzimidazolyl)]-*Co*β-hydroxocobamide; CAS Reg. No. 13422-51-0 (anhydrous). Cobinamide dihydroxide monohydrate, dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole; cobinamide dihydroxide monohydrate, dihydrogen phosphate (ester), mono(inner salt) 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole; *Co*α-[α-(5,6-dimethylbenzimidazolyl)]-*Co*β-hydroxocobamide monohydrate; CAS Reg. No. 13422-52-1 (monohydrate).

Other names. Vitamin B_{12a} for anhydrous Hydroxocobalamin and Vitamin B_{12b} for Hydroxocobalamin hydrate.

Description. Dark red crystals or a red, crystalline powder; odourless.

Solubility. Sparingly soluble in water and ethanol (~750 g/l) TS; practically insoluble in acetone R.

Category. Antianaemia drug.

Storage. Hydroxocobalamin should be kept in a tightly closed container, protected from light, and stored in a cool place.

Labelling. The designation on the container should state whether the substance is in the anhydrous or hydrated form.

Additional information. In aqueous solution, Hydroxocobalamin exists as hydroxocobamide in equilibrium with the hydrated ionic form. The anhydrous form of Hydroxocobalamin is very hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Hydroxocobalamin contains not less than 96.0% and not more than 102.0% of $C_{62}H_{89}CoN_{13}O_{15}P$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 40 µg/ml solution in pH 4.5 acetate buffer TS, when observed between 230 nm and 550 nm, exhibits 3 maxima at about 274 nm, 351 nm, and 525 nm; the ratio of the absorbance of a 1-cm layer at 525 nm to that at 351 nm is about 0.34, and the ratio of the absorbance at 274 nm to that at 351 nm is about 0.80.

B. Heat cautiously about 2 mg in a porcelain crucible with a few drops of sulfuric acid (~1760 g/l) TS until a faintly bluish residue is produced. Cool, add 0.05 ml of water and then a few drops of a saturated solution of ammonium thiocyanate R; a blue-green colour is produced.

C. Place about 2 mg in a 100-ml glass-stoppered flask, dissolve in 2 ml of water and add 5 ml of phosphoric acid (~1440 g/l) TS. Insert in the flask a flat-bottomed glass tube 1 cm in diameter and 2 cm long, containing 1 ml of lithium carbonate/trinitrophenol TS. Close the flask and expose it for 4 hours to a bright light; the colour of the reagent in the glass tube remains unchanged (distinction from cyanocobalamin).

Loss on drying. Dry Hydroxocobalamin hydrate at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 2 hours; it loses between 140 mg/g and 180 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 8.0–10.0.

Other cobalamins. Carry out the test as described under 1.14.3 Column chromatography, shaking 20 g of diethylaminoethylcellulose R with 200 ml of sodium hydroxide (0.5 mol/l) VS, dilute with water to obtain a homogeneous suspension, allow to settle, and discard the supernatant liquid. Using a suitable filter, wash with water until the washings are free from alkali, then transfer the adsorbent to a tube, length 22 cm, diameter 1.2 cm, and provided with a stopcock. Allow to settle and tap the tube until the height of the adsorbent is about 14 cm. Wash with water until the pH of the eluate is the same as that of the water.

Similarly prepare a second column, slurrying carboxymethylcellulose R with hydrochloric acid (0.5 mol/l) VS, dilute with water, allow to settle, and discard the supernatant liquid. Using a suitable filter, wash with water until the washings are free from acid, then transfer the adsorbent to a tube, length 22 cm, diameter 1.2 cm, and provided with a stopcock. Allow to settle and tap the tube until the pH of the eluate is the same as that of the water.

Cover each column with a plug of glass wool and allow to drain until only a small amount of water remains above the adsorbents.

Place the column of diethylaminoethylcellulose above the other column so that the effluent runs into the carboxymethylcellulose.

Weigh accurately about 0.05 g of the substance to be examined, dissolve it in 20 ml of water, and acidify with sufficient hydrochloric acid (~70 g/l) TS to obtain a pH of 4.0. Introduce this solution to the diethylaminoethylcellulose column and allow it to run through both columns, rejecting the first colourless eluate. Elute with water, the pH of which has previously been adjusted to 4.0 with hydrochloric acid (~70 g/l) TS. Collect the coloured eluate into a 50-ml volumetric flask and adjust to volume with water. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 361 nm and calculate the content of other cobalamins in mg/g, using the absorptivity value of 20.7 ($A^{1\%}_{1\,cm}$ = 207); not more than 30 mg/g.

Acidic impurities. Elute the diethylaminoethylcellulose column from the above test for other cobalamins with sodium chloride (10 g/l) TS, collecting 50 ml of eluate. Measure the absorbance of this solution in a 1-cm layer at the maximum between 351 nm and 361 nm, and calculate the content of acidic impurities in mg/g, using the absorptivity value of 19.0 ($A^{1\%}_{1\,cm}$ = 190); not more than 30 mg/g.

Assay

- The solutions must be protected from light throughout the assay.

Dissolve about 20 mg, accurately weighed, in sufficient acetate buffer, pH 4.5, TS to produce 500 ml. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 351 nm and calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$, using the absorptivity value of 19.5 ($A^{1\%}_{1\,cm} = 195$).

Additional requirements for Hydroxocobalamin for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.4 IU of endotoxin RS per mg.

HYDROXYETHYLCELLULOSUM
HYDROXYETHYLCELLULOSE

Chemical name. Cellulose 2-hydroxyethyl ether; CAS Reg. No. 9004-62-0.

Description. A white or yellowish white powder or granules; odourless or almost odourless.

Solubility. Soluble in hot and cold water forming a colloidal solution; practically insoluble in acetone R, ethanol (~750 g/l) TS, ether R, and toluene R.

Category. Stabilizer; suspending agent.

Storage. Hydroxyethylcellulose should be kept in a well-closed container.

Labelling. The designation on the container of hydroxyethylcellulose should state its viscosity.

Additional information. Hydroxyethylcellulose may contain suitable anticaking agents. After drying, it is hygroscopic.

Requirements

Definition. Hydroxyethylcellulose is a partially substituted poly(hydroxyethyl) ether of cellulose.

Identity tests

A. Disperse 1 g of dried Hydroxyethylcellulose in 50 ml of carbon-dioxide-free water R. After 10 minutes, dilute to 100 ml with the same solvent and stir until completely dissolved. While stirring, heat 10 ml on a water-bath (keep the remaining solution for test B, for "pH value", and for "Reducing substances"); no cloudiness appears above 50 °C and no precipitate is formed.

B. Place 1 ml of the above solution onto a glass plate and allow to evaporate; a thin film is formed.

C. Dissolve 5 mg in 1 ml of water, add 1 ml of phenol (50 g/l) TS and 5 ml of sulfuric acid (~1760 g/l) TS, shake carefully, and allow to cool; a red colour develops.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 50 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 100 mg/g.

pH value. pH of the solution prepared in identity test A, 5.5–8.5.

Reducing substances. Add 5 ml of water to 5 ml of the solution prepared in identity test A, then add 15 drops of sulfuric acid (0.5 mol/l) VS and 1.5 ml of potassium permanganate (0.002 mol/l) VS. Heat the mixture to 50 °C for not less than 5 minutes; the colour of the solution remains unchanged.

HYDROXYPROPYLCELLULOSUM
HYDROXYPROPYLCELLULOSE

Chemical name. Cellulose 2-hydroxypropyl ether; CAS Reg. No. 9004-64-2.

Description. A white or yellowish white powder; odourless.

Solubility. Soluble in cold water, ethanol (~750 g/l) TS, methanol R, and propylene glycol R giving colloidal solutions; practically insoluble in hot water.

Category. Film-coating agent; tablet binder; granulating agent; viscosity-increasing agent; suspending agent.

Storage. Hydroxypropylcellulose should be kept in a well-closed container.

Labelling. The designation on the container of Hydroxypropylcellulose should state its viscosity.

Additional information. Hydroxypropylcellulose is hygroscopic after drying.

Requirements

Definition. Hydroxypropylcellulose is a cellulose having some of the hydroxyl groups in the form of 2-hydroxypropyl ether.

Identity tests

A. While stirring, add 1 g of dried Hydroxypropylcellulose to 50 ml of carbon-dioxide-free water R heated to 90 °C. Allow to cool, dilute to 100 ml with the same solvent, and stir until completely dissolved. While stirring, heat 10 ml on a water-bath (keep the remaining solution for test B and for "pH value"); above 40 °C a cloudy solution or a flocculent precipitate is formed, and on cooling the solution becomes clear.

B. Place 1 ml of the above solution onto a glass plate and allow to evaporate; a thin film is formed.

C. Dissolve without heating 0.2 g in 15 ml of sulfuric acid (~1125 g/l) TS. Pour the solution with stirring into 100 ml of ice-water, and dilute to 250 ml with ice-water. While cooling in ice-water, mix thoroughly in a test-tube 1 ml of the prepared solution with 8 ml of sulfuric acid (~1760 g/l) TS, added drop by drop. Heat in a water-bath for exactly 3 minutes and immediately cool in ice-water. While cold, add carefully 0.6 ml of triketohydrindene/sodium metabisulfite TS, mix well, and allow to stand at 25 °C; a pink colour is immediately produced, which changes to violet within 100 minutes.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 70 mg/g.

pH value. pH of the solution prepared for identity test A, 5.0–8.5.

HYPROMELLOSUM
HYPROMELLOSE

Chemical name. Cellulose 2-hydroxypropyl methyl ether; CAS Reg. No. 9004-65-3.

Other name. Hydroxypropylmethylcellulose.

Description. A white or creamy white, fibrous or granular powder; odourless or almost odourless.

Solubility. Soluble in cold water, forming a clear or viscous, colloidal solution; practically insoluble in ethanol (~750 g/l) TS and ether R; soluble in mixtures of methanol R and dichloromethane R.

Category. Suspending agent; tablet binder; viscosity-increasing agent.

Storage. Hypromellose should be kept in a well-closed container.

Labelling. The designation on the container of Hypromellose should state its viscosity.

Requirements

Definition. Hypromellose is a propylene glycol ether of methylcellulose.

Identity tests

A. Disperse 1 g of Hypromellose in 100 ml of water, allow the beaker to stand until the dispersion becomes transparent and mucilaginous (about 5 hours), swirl the beaker, and stir until completely dissolved. To two 10-ml aliquots (keep the remaining solution for test B and for "pH value") add an equal volume of either sodium hydroxide (1 mol/l) VS or hydrochloric acid (1 mol/l) VS; both mixtures remain unchanged.

B. Place 1 ml of the above solution onto a glass plate and allow to evaporate; a thin film is formed.

C. Add 1 g of powdered Hypromellose to 100 ml of boiling water and stir the mixture; a slurry is formed, but the powdered material does not dissolve. Cool the slurry to 20 °C and stir; the resulting liquid is a clear or opalescent, mucilaginous colloidal mixture.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3, adding 1 ml of hydroxy-

lamine hydrochloride (200 g/l) TS to the solution of the residue; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 15 mg/g.

Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 50 mg/g.

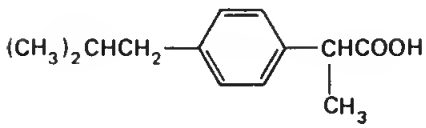
pH value. pH of the solution prepared for identity test A, 5.0–8.0.

IBUPROFENUM
IBUPROFEN

Molecular formula. $C_{13}H_{18}O_2$

Relative molecular mass. 206.3

Graphic formula.



Chemical name. *p*-Isobutylhydratropic acid; α-methyl-4-(2-methylpropyl) benzeneacetic acid; 2-(*p*-isobutylphenyl)propionic acid; CAS Reg. No. 15687-27-1.

Description. Colourless crystals or a white, crystalline powder; odour, characteristic.

Solubility. Practically insoluble in water; soluble in 1.5 parts of ethanol (~750 g/l) TS, in 2 parts of ether R, and in 1.5 parts of acetone R.

Category. Analgesic; anti-inflammatory.

Storage. Ibuprofen should be kept in a well-closed container.

Requirements

Definition. Ibuprofen contains not less than 98.5% and not more than 100.5% of $C_{13}H_{18}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ibuprofen RS or with the *reference spectrum* of ibuprofen.

B. The absorption spectrum of a 0.25 mg/ml solution in sodium hydroxide (0.1 mol/l) VS, when observed between 220 nm and 350 nm, is qualitatively similar to that of a 0.25 mg/ml solution of ibuprofen RS in sodium hydroxide (0.1 mol/l) VS (maxima occur at about 264 nm and 273 nm, and a shoulder at about 259 nm). The absorbances of the solutions at the respective maxima do not differ from each other by more than 3%. The absorbance of a 1-cm layer at the wavelengths of the main maxima at 264 nm and 273 nm are about 0.46 and 0.39, respectively (preferably use 2-cm cells for the measurement and calculate the absorbances of 1-cm layers).

C. Melting temperature, about 76 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 5.0 mg/g.

Related substances

A. Carry out the test as described under 1.14.5 Gas chromatography, using a solution of the test substance prepared as follows: To 0.10 g add diazomethane TS until effervescence ceases and a persistent yellow colour is produced. Remove the solvent in a current of nitrogen, warming gently if necessary, and dissolve the residue in 2 ml of chloroform R. Use this solution in procedures A1 and A2.

In procedure A1 use a glass column 1.8 m long and 3.0 mm in internal diameter packed with an adsorbent composed of 1 g of macrogol 20M R and 9 g of acid-washed, silanized kieselguhr R3, and maintained at 135 °C. Use nitrogen R as the carrier gas and a flame ionization detector. In this system, determine only those impurities with a relative retention time of less than 2.5, taking the retention time of ibuprofen as 1.0. The ratio of the total area of the peaks due to these impurities to the area under the peak of ibupro-

fen does not exceed 0.010, and the corresponding ratio for the peak of any individual impurity does not exceed 0.003.

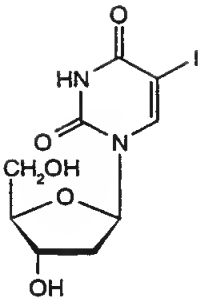
In procedure A2 use a glass column 1.8 m long and 3.0 mm in internal diameter packed with an adsorbent composed of 0.5 g of methyl silicone gum R, 0.2 g of cyanoethylmethyl silicone gum R and 9.3 g of acid-washed, silanized kieselguhr R4 and maintained at 170 °C. Use nitrogen R as the carrier Gas and a flame ionization detector. In this system, determine only those impurities with a relative retention time of between 1.5 and 6.0, taking the retention time of ibuprofen as 1.0. The ratio of the total area of the peaks due to these impurities to the area under the peak of ibuprofen does not exceed 0.010.

The sum of the ratios found in procedures A1 and A2 does not exceed 0.015.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 15 volumes of 1-hexane R, 5 volumes of ethyl acetate R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in chloroform R containing (A) 100 mg of the test substance per ml and (B) 1 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray very lightly with a 10 mg/ml solution of potassium permanganate R in sulfuric acid (~100 g/l) TS, heat at 120 °C for 20 minutes, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 100 ml of ethanol (~750 g/l) TS previously neutralized to phenolphthalein/ethanol TS, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 20.63 mg of $C_{13}H_{18}O_2$.

IDOXURIDINUM
IDOXURIDINE



$C_9H_{11}IN_2O_5$

Relative molecular mass. 354.1

Chemical name. 2′-Deoxy-5-iodouridine; CAS Reg. No. 54-42-2.

Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water, ethanol (~750 g/l) TS and hydrochloric acid (~70 g/l) TS; freely soluble in sodium hydroxide (~80 g/l) TS.

Category. Anti-infective agent.

Storage. Idoxuridine should be kept in a well-closed container, protected from light.

Labelling. The designation Idoxuridine for sterile non-injectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

Additional information. Melting temperature, about 180 °C, with decomposition.

Requirements

Idoxuridine contains not less than **98.0%** and not more than **101.0%** of $C_9H_{11}IN_2O_5$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from idoxuridine RS or with the *reference spectrum* of idoxuridine.

B. Dissolve about 2 mg in 50 ml of sodium hydroxide (0.01 mol/l) VS; the absorption spectrum, when observed between 230 nm and 350 nm, exhibits a maximum at about 279 nm.

C. See the test described below under "Related substances". The principal spot obtained with solution D corresponds in position, appearance, and intensity to that obtained with solution E.

D. Heat about 5 mg in a test-tube over an open flame; violet vapours are evolved.

Specific optical rotation. Use a 0.10 g/ml solution in sodium hydroxide (1 mol/l) VS and determine the rotation immediately after preparation. Calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +28\,°$ to $+32\,°$.

Iodine and iodide. For solution (A) dissolve 0.10 g in a mixture of 20 ml of water and 5 ml of sodium hydroxide (~80 g/l) TS, and immediately add 5 ml of sulfuric acid (~100 g/l) TS. Cool in an ice-bath, allow to stand for 10 minutes, shaking occasionally, and filter. For solution (B) dissolve 0.111 g of potassium iodide R in sufficient water to produce 1000 ml. To 1.0 ml of this solution add 19 ml of water, 5 ml of sodium hydroxide (~80 g/l) TS, and 5 ml of sulfuric acid (~100 g/l) TS, mix, and filter. Transfer both filtrates from solutions A and B to 103 separate comparison tubes, to each add 10 ml of dichloromethane R and 3 drops of potassium iodate (0.05 mol/l) VS, shake for 30 seconds, and allow to stand. The colour in the dichloromethane layer of solution A is not more intense than that produced with solution B when compared as described under 1.11 Colour of liquids.

Solution in alkali. A solution of 0.10 g in 10 ml of sodium hydroxide (1 mol/l) VS is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry about 1 g to constant mass at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 5 volumes of 2-propanol R, 4 volumes of dichloromethane R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl

of each of 3 solutions in a mixture of 5 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS containing (A) 40 mg of Idoxuridine per ml, (B) 0.20 mg of Idoxuridine per ml, (C) 0.10 mg of Idoxuridine per ml, (D) 4 mg of Idoxuridine per ml, and (E) 4 mg of idoxuridine RS per ml. After removing the plate from the chromatographic chamber, dry it in a current of cold air and repeat the development. After removing the plate following the second development from the chromatographic chamber, dry it in a current of cold air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Not more than 3 such spots are more intense than the spot obtained with solution C (0.25%).

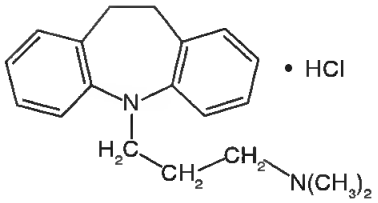
Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method B, determining the end-point potentiometrically.

Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 35.41 mg of $C_9H_{11}IN_2O_5$.

Additional requirement for Sterile non-injectable Idoxuridine

Complies with 3.2.1 Test for sterility of non-injectable preparations.

IMIPRAMINI HYDROCHLORIDUM
IMIPRAMINE HYDROCHLORIDE



$C_{19}H_{24}N_2,HCl$

Relative molecular mass. 316.9

Chemical name. 5-[3-(Dimethylamino)propyl]-10,11-dihydro-5*H*-dibenz[*b,f*] azepine monohydrochloride; 10,11-dihydro-*N,N*-dimethyl-5*H*-dibenz[*b,f*] azepine-5-propanamine monohydrochloride; CAS Reg. No. 113-52-0.

Other name. Imizine.

Description. A white or slightly yellowish, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water, ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Psychotherapeutic drug.

Storage. Imipramine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Imipramine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Imipramine hydrochloride contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{19}H_{24}N_2$,HCl, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from imipramine hydrochloride RS or with the *reference spectrum* of imipramine hydrochloride.

B. Dissolve 2 mg in 2.0 ml of water and add 2 ml of nitric acid (~1000 g/l) TS; an intense blue colour is produced.

C. Dissolve 0.05 g in 3 ml of water and add 0.05 ml of quinhydrone/methanol TS; no red colour develops within 15 minutes.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 170–174 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. Dissolve rapidly by shaking and triturating with a glass rod 1 g in 10 ml of carbon-dioxide-free water R; the solution is clear. Immediately dilute half the solution with an equal volume of water; it is not

more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids. (Keep the remaining solution for the "pH value".)

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of the solution prepared for the "Clarity and colour of solution" measured immediately after preparation, 3.6–5.0.

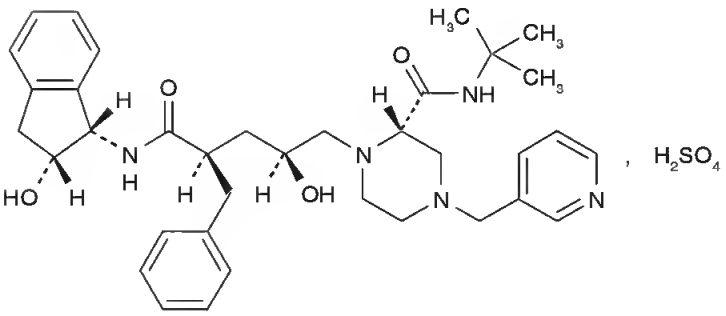
Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 5 volumes of hydrochloric acid (~250 g/l) TS, 5 volumes of water, 35 volumes of glacial acetic acid R, and 55 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of three solutions in methanol R prepared immediately before use containing (A) 25 mg of Imipramine hydrochloride per ml, (B) 0.05 mg of Imipramine hydrochloride per ml, and (C) 0.05 mg of iminodibenzyl R per ml. After removing the plate from the chromatographic chamber, allow it to dry for 5 minutes, spray with a solution of 0.5 g of potassium dichromate R dissolved in 100 ml of a mixture of 4 volumes of water and 1 volume of sulfuric acid (~1760 g/l) TS. Examine the chromatogram immediately in daylight.

Any spot obtained with solution A, other than the principal spot which should be blue in colour and the spot corresponding to iminodibenzyl as obtained with solution C, is not more intense than the spot obtained with solution B. Furthermore, any spot obtained with solution A corresponding to iminodibenzyl is not more intense than the spot obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 31.69 mg of $C_{19}H_{24}N_2,HCl$.

INDINAVIRI SULFAS
INDINAVIR SULFATE



$C_{36}H_{47}N_5O_4,H_2O_4S$

Relative molecular mass. 711.9

Chemical name. (2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide sulfate; CAS Reg. No. 157810-81-6.

Description. A white or almost white powder.

Solubility. Freely soluble in water, soluble in methanol.

Category. Antiretroviral (protease inhibitor).

Storage. Indinavir sulfate should be kept in a tightly closed container, protected from light.

Additional information. Indinavir sulfate occurs as the monoethanolate which is hygroscopic. It converts to the hydrate upon loss of ethanol and exposure to moist air.

Requirements

Indinavir sulfate contains not less than **98.5%** and not more than **101.0%** of $C_{36}H_{47}N_5O_4,H_2O_4S$ calculated on anhydrous, ethanol free basis.

Identity tests

- Either tests A, B and D, or tests C and D may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of indinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of indinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120 °C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 0.100 mg/ml solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 260 nm; the specific absorbance ($A_{1\,cm}^{1\%}$) is between 56 and 65.

C. Dissolve 0.1 g in 10 ml of water, add 2 ml of sodium hydroxide (~80 g/l) TS and shake. Filter the resulting precipitate and wash with two 3-ml quantities of water. Dry the washed precipitate for one hour at 105 °C. Using the dried precipitate thus obtained, carry out the examination as described under "1.7 Spectrophotometry in the infrared region". The infrared absorption spectrum is concordant with the spectrum obtained from indinavir RS or with the *reference spectrum* of indinavir.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 10.0 mg/ml solution and calculate with reference to the anhydrous and ethanol free substance; $[\alpha]_D^{20\,°C}$ = +27 ° to +31 °.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g of the substance; the water content is not more than 15 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 2.8–3.2.

Ethanol content. Determine by 1.14.5 Gas chromatography, using static head-space injection. Use a fused-silica capillary or wide bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with macrogol 20M R (film thickness: 0.25 µm).

As detector use a flame ionization detector.

Use nitrogen for chromatography R or helium R as the carrier gas at an appropriate pressure and a split ratio 1:5 with a linear velocity of about 35 cm/sec.

The following head-space injection conditions may be used:

Equilibration temperature (°C)	80
Equilibration time (min)	60
Transfer line temperature (°C)	85
Pressurization time (s)	30
Injection volume (ml)	1

Maintain the temperature of the column at 30 °C for 7 min, then raise the temperature at a rate of 35 °C per min to 180 °C and maintain for 10 min, maintaining the temperature of the injection port at 140 °C and that of the flame ionization detector at 250 °C.

Test solution. Dissolve 0.200 g of the test substance in purified water and dilute to 20.0 ml with the same solvent. Introduce 5.0 ml of this solution and 1.0 ml of purified water into a headspace vial. Prepare two more vials.

Reference solutions. Add 0.200 g of ethanol R to purified water and dilute to 200.0 ml with the same solvent. Transfer respectively 2.0 ml, 3.0 ml and 4.0 ml in separate headspace injection vials and bring the volume to 6.0 ml with purified water.

Blank solution. Introduce 6.0 ml of purified water into a headspace vial.

Analyse the blank solution and then alternatively three times the test solution and the three reference solutions.

The test is not valid unless the relative standard deviation on the areas of the peaks obtained from the test solutions is not more than 5%.

Calculate the ethanol content by using the results obtained with the test solution and with the reference solutions; the ethanol content is not less than 50 mg/g and not more than 80 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 30 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 65 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 25 volumes of purified water.

Prepare the phosphate buffer pH 7.5 by dissolving 1.0 g of anhydrous disodium hydrogen phosphate R in 50 ml of purified water, adjust the pH to 7.5 by adding phosphoric acid (~105 g/l) TS and dilute it to 100 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–5	93	7	Isocratic
5–25	93 to 20	7 to 80	Linear gradient
25–30	20	80	Isocratic
30–35	20 to 93	80 to 7	Return to the initial conditions
35–45	93	7	Isocratic re-equilibration

Prepare the following solutions. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 2 µg of Indinavir sulfate per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 2 ml of sulfuric acid (190 g/l), heat carefully in a boiling water bath for 10 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 40 °C.

Inject 20 µl of solution (3). The test is not valid unless the resolution factor between the two major peaks, with a retention time between 15 and 20 min, is not less than 3.5. If necessary adjust the amount of acetonitrile in mobile phase A, or adjust the gradient program.

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than the area of the principal peak obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

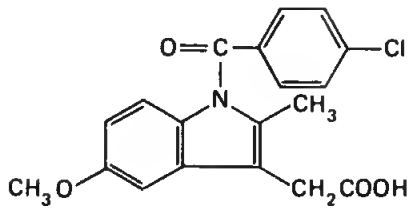
Assay. Dissolve 0.300 g, accurately weighed, in 50 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 35.59 mg of $C_{36}H_{47}N_5O_4,H_2O_4S$; calculate with reference to the anhydrous and ethanol free substance.

INDOMETACINUM
INDOMETACIN

Molecular formula. $C_{19}H_{16}ClNO_4$

Relative molecular mass. 357.8

Graphic formula.



Chemical name. 1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid; 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid; CAS Reg. No. 53-86-1.

Description. A white or a pale yellow, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS and ether R.

Category. Analgesic; anti-inflammatory.

Storage. Indometacin should be kept in a well-closed container, protected from light.

Additional information. Indometacin exhibits polymorphism. The polymorph specified in the monograph corresponds to the crystal form of indometacin RS.

Requirements

Definition. Indometacin contains not less than 98.0% and not more than 101.0% of $C_{19}H_{16}ClNO_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum obtained from the solid state of the test substance is concordant with the spectrum obtained from indometacin RS or with the *reference spectrum* of indometacin (confirmation of polymorphic form).

B. Dissolve 0.1 g in 100 ml of water containing 0.5 ml of sodium hydroxide (1 mol/l) VS. To a 1 ml-portion add 1 ml of freshly prepared sodium nitrite (1 g/l) TS and allow to stand for 5 minutes. Add 0.5 ml of sulfuric acid (~1760 g/l) TS; a deep yellow colour is produced. To another 1-ml portion add 1 ml of sodium nitrite (1 g/l) TS, and allow to stand for 5 minutes. Add 0.5 ml of hydrochloric acid (~420 g/l) TS; a green colour is produced.

C. Melting temperature, about 160 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

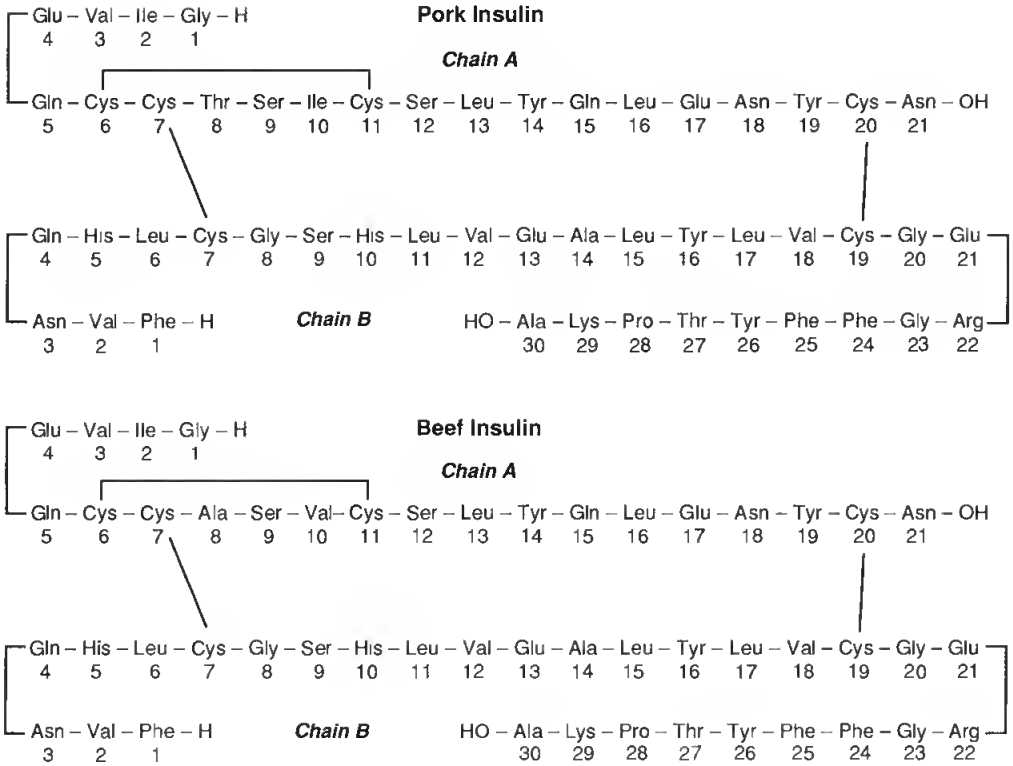
Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and preparing the slurry in sodium dihydrogen phosphate (45 g/l) TS. As the mobile phase, use a mixture of 7 volumes of ether R and 3 volumes of light petroleum R. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 20 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.33 g, accurately weighed, in 75 ml of acetone R through which nitrogen R free from carbon dioxide has previously been passed for 15 minutes. Maintain a constant stream of nitrogen through the solution and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS using phenolphthalein/ethanol TS as indicator or determining the endpoint potentiometrically. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 35.78 mg of $C_{19}H_{16}ClNO_4$.

INSULINUM
INSULIN



$C_{256}H_{381}N_{65}O_{76}S_6$ (pork)
$C_{254}H_{377}N_{65}O_{75}S_6$ (beef)

Definition. Insulin is the natural antidiabetic hormone obtained from pork or beef pancreas and purified.

Chemical name. [Pork] Insulin; porcine insulin; CAS Reg. No. 12584-58-6. [Beef] Insulin; bovine insulin; CAS Reg. No. 11070-73-8.

Description. A white or almost white powder; odourless.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, and ether R; dissolves in dilute solutions of mineral acids and alkali hydroxides.

Category. Antidiabetic agent.

Storage. Insulin should be kept in a tightly closed container, protected from light, and stored at a temperature not below –20 °C.

Labelling. The designation on the container should state the animal source of the insulin; expiry date.

Additional information. Insulin is hygroscopic; it degrades in solutions of alkali hydroxides. Attention should be paid to the microbiological quality since Insulin is of natural origin.

The quality of insulin, which represents a unique case among preparations in the WHO Model List of Essential Drugs, cannot be adequately controlled by methods adopted for *The International Pharmacopoeia*. Therefore, it is advised to refer to methods described in other pharmacopoeias, such as those of China, Europe, Japan, UK, and USA.

IODUM
IODINE

Molecular formula. I_2

Relative molecular mass. 253.8

Chemical name. Iodine; CAS Reg. No. 7553-56-2.

Description. Heavy, greyish black plates or granules, having a metallic lustre.

Solubility. Very slightly soluble in water; soluble in ethanol (~750 g/l) TS; freely soluble in carbon tetrachloride R, carbon disulfide R, and ether R.

Category. External antiseptic.

Storage. Iodine should be kept in a tightly closed container, preferably made of glass and provided with a glass stopper.

Additional information. Iodine volatilizes slowly at room temperature giving violet, irritant vapours.

Requirements

Definition. Iodine contains not less than 99.5% and not more than 100.5% of I.

Identity tests

A. Dissolve 0.05 g in 10 ml of ethanol (~750 g/l) TS; the colour of the solution is reddish brown. Dissolve 0.05 g in 10 ml of carbon tetrachloride R; the colour of the solution is violet.

B. To a saturated solution in water add starch TS; a blue colour is produced. Boil the mixture for a short time; the solution loses its colour, but on cooling the colour reappears.

Chlorides and bromides. Triturate 1.5 g with 10 ml of water, filter, wash the filter and dilute the filtrate to 15 ml with water. To the solution add 0.5 g of zinc R powder. When the solution has become decolourized, filter and wash the filter with sufficient water to adjust the volume of the filtrate to 20 ml. To 5 ml of the filtrate (keep the remaining filtrate for the test for cyanides) add 1.5 ml of ammonia (~260 g/l) TS and 3 ml of silver nitrate (40 g/l) TS, filter, wash the filter with sufficient water to adjust the volume of the filtrate to 10 ml, add 1.5 ml of nitric acid (~1000 g/l) TS and allow to stand for 1 minute. Any opalescence in the solution is not more intense than that obtained from a solution simultaneously prepared by mixing 10.75 ml of water, 0.25 ml of hydrochloric acid (0.01 mol/l) VS, 0.2 ml of nitric acid (~130 g/l) TS, and 0.3 ml of silver nitrate (40 g/l) TS; this indicates that the content of chlorides and bromides is not more than 0.25 mg/g.

Cyanides. To 5 ml of the filtrate obtained in the test for chlorides and bromides add 0.2 ml of ferrous sulfate (15 g/l) TS and 1 ml of sodium hydroxide (~80 g/l) TS. Heat for a few minutes and acidify with hydrochloric acid (~70 g/l) TS; no blue or green colour is produced.

Non-volatile residue. Place about 1 g, accurately weighed, in a porcelain dish and heat on a water-bath until the iodine has volatilized. Dry the residue for 1 hour at 105 °C and weigh; not more than 1.0 mg/g.

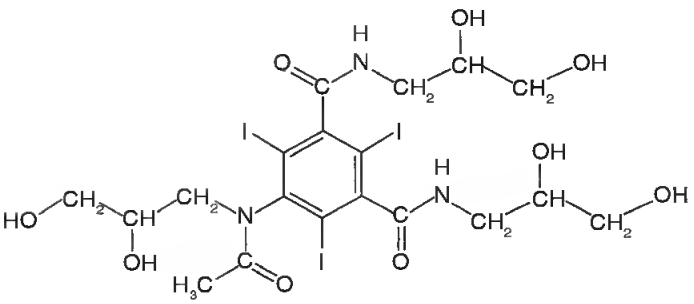
Assay. Dissolve about 0.5 g of finely powdered test substance, accurately weighed, in a solution of 1 g of potassium iodide R in 5 ml of water. Dilute with water to about 50 ml, add 1 ml of hydrochloric acid (~70 g/l) TS and titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 12.69 mg of I.

Additional requirement for Iodine for parenteral use

Complies with the monograph for "Parenteral preparations".

IOHEXOLUM

IOHEXOL



$C_{19}H_{26}I_3N_3O_9$

Relative molecular mass. 821.1

Chemical name. *N,N'*-Bis(2,3-dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl)-acetamido]-2,4,6-triiodoisophthalamide; 5-[acetyl(2,3-dihydroxypropyl)amino]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide; CAS Reg. No. 66108-95-0.

Description. A white to greyish, amorphous powder; odourless.

Solubility. Very soluble in water and methanol R.

Category. Radiocontrast medium.

Storage. Iohexol should be kept in a well-closed container, protected from light.

Additional information. Melting range, 177–187 °C.

Requirements

Iohexol contains not less than **98.5%** and not more than the equivalent of **101.5%** of $C_{19}H_{26}I_3N_3O_9$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from iohexol RS or with the *reference spectrum* of iohexol.

B. The absorption spectrum of a 10 µg/ml solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 245 nm; the absorbance of a 1-cm layer at the maximum wavelength is about 0.36.

C. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

D. Heat 0.05 g in a suitable crucible; violet vapours are evolved.

Aluminium. Place in three separatory funnels 2 ml of a freshly prepared aluminium standard (10 µg Al/ml) TS, 10 ml of a solution containing 0.5 g of Iohexol per ml, and 10 ml of water to serve as a blank. To each funnel add 5 ml of ammonium chloride buffer, pH 10.5, TS and dilute to 25 ml with water. To every funnel add 5 ml of 8-hydroxyquinoline/chloroform TS and shake for 2 minutes. Allow the phases to separate and measure the absorbance of the extracts against the extracted blank solution at a maximum at about 395 nm; the aluminium content is not more than 4 µg/g.

Copper. Place in three separatory funnels 0.25 ml of a freshly prepared copper standard (10 µg/ml Cu) TS, 10 ml of a solution containing 0.5 g of Iohexol per ml, and 15 ml of water to serve as a blank. To each funnel add 1 ml of ammonium pyrrolidinedithiocarbamate (10 g/l) TS and 5 ml of acetate buffer, pH 4.5, TS and dilute to 25 ml with water. To each funnel add 5 ml of isobutyl methyl ketone R and shake for 2 minutes. Allow the phases to separate and measure the absorbance of the extracts against the extracted blank solution at a maximum at about 435 nm; the copper content is not more than 0.5 µg/g.

Halides. Dissolve 5 g in 20 ml of water and titrate with silver nitrate (0.001 mol/l) VS, determining the endpoint potentiometrically, using silver/silver chloride electrodes. Repeat the procedure without the Iohexol being examined and make any necessary corrections. Each ml of silver nitrate (0.001 mol/l) VS is equivalent to 0.1269 mg of I; the content of halides, calculated as iodides, does not exceed 20 µg/g.

Colour of solution. Dissolve 6.47 g in sufficient water to produce 10 ml (apply a correction for any presence of water). The concentration of the solution is 64.7%, equivalent to 300 mg of 1 per ml. Filter through a Millipore filter 0.22 µm, and measure the absorbance in a 1-cm cell using water as a blank at about 400 nm, 420 nm, and 450 nm. The absorbance is not greater than 0.200, 0.050, and 0.025, respectively.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.2 g; the water content is not more than 50 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 50 volumes of 1-butanol R, 11 volumes of acetic acid (~300 g/l) TS, and 25 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of four solutions in methanol R containing (A) 10 mg of Iohexol per ml, (B) 10 mg of iohexol RS per ml, (C) 20 mg of Iohexol per ml, and (D) 40 µg of Iohexol per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D.

Primary aromatic amines

- The solutions must be protected from light throughout the procedure.

To about 0.2 g, accurately weighed, add 15 ml of water, mix to dissolve, and allow to stand in an ice-bath for 5 minutes. Add 1.5 ml of hydrochloric acid (~250 g/l) TS and 2 ml of sodium nitrite (10 g/l) TS, mix, and return the flask to the ice-bath for exactly 4 minutes. Add 1 ml of sulfamic acid (50 g/l) TS and again place the flask in the ice-bath for exactly 1 minute. Remove the flask from the bath, add 0.5 ml of freshly prepared *N*-(1-naphthyl)ethylenediamine hydrochloride/propylene glycol TS, mix, and dilute to 25 ml with water. Within 20 minutes measure the absorbance of a 5-cm layer at the maximum at about 495 nm against a solvent cell containing a solution prepared by treating 15 ml of water in a similar manner; the absorbance does not exceed 0.21.

Assay. Carry out the combustion as described under 2.4 Oxygen flask method for iodine, using about 7.5 mg, accurately weighed. Titrate the liberated iodine with sodium thiosulfate (0.01 mol/l) VS.

Each ml of sodium thiosulfate (0.01 ml/l) VS is equivalent to 0.4562 mg of $C_{19}H_{26}I_3N_3O_9$.

IPECACUANHAE RADIX
IPECACUANHA ROOT

Description. Odour, slight; taste, bitter, nauseous and acrid.

Category. Expectorant; emetic.

Storage. Ipecacuanha root should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, the powder of Ipecacuanha root is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Ipecacuanha root consists of the dried rhizome and roots of *Cephaëlis ipecacuanha* (Brotero) A. Richard (Fam. Rubiaceae) or of *Cephaëlis acuminata* Karsten, or of a mixture of both species. The principal alkaloids are emetine and cephaëline.

Ipecacuanha root contains not less than 2.0% of total alkaloids, calculated as emetine.

Macroscopic characteristics

Cephaëlis ipecacuanha. Dark brick-red to very dark brown, somewhat tortuous root, seldom more than 15 cm long or 6 mm thick; the root is closely annulated externally, having rounded ridges completely encircling it; the fracture is short in the bark and splintery in the wood; a transversely cut surface shows a wide greyish bark and a small uniformly dense wood. The rhizomes are short lengths attached to roots; they are cylindrical, up to 2 mm in diameter, finely wrinkled longitudinally, and with pith occupying approximately one-sixth of the whole diameter.

Cephaëlis acuminata. In general it resembles the root of *Cephaëlis ipecacuanha*, but differs in the following particulars: often up to 9 mm thick; external surface greyish brown or reddish brown with transverse ridges at intervals of about 1–3 mm; the ridges are about 0.5–1 mm wide, extending about half-way round the circumference and fading at the extremities into the general surface level.

Microscopic characteristics

Cephaëlis ipecacuanha. A transverse section of the root shows a narrow, brown cork layer of thin-walled polyhedral, tubular cells and a wide parenchymatous zone of phelloderm; the latter contains abundant starch, consisting of simple granules and compound granules of 2–8 components, the individual granules being oval, rounded, or roughly hemispherical, and seldom more than 15 µm in diameter; the phloem is present as a narrow unlignified zone; the xylem is dense, consisting mainly of narrow tracheids intermixed with a smaller proportion of vessels, both with numerous bordered pits in their lateral walls, the vessel element having simple circular perforations; crystal cells, each containing a bundle of raphides, 30–80 µm long, occur in the parenchymatous regions. A transverse section through an internode of the rhizome shows several layers of thin-walled cork, a somewhat collenchymatous cortex, a pericycle containing groups of large, distinctly pitted sclereids, a narrow ring of phloem, and a wide ring of xylem surrounding a pith composed of thin-walled, pitted, parenchymatous cells.

Cephaëlis acuminata. Similar to *Cephaëlis ipecacuanha* except that the individual starch granules may be up to 22 µm in diameter.

Identity test
Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 93 volumes of chloroform R, 6.5 volumes of methanol R, and 0.5 volumes of ammonia (~260 g/l) TS as the mobile phase, and allow the solvent front to ascend only 10 cm above the line of application. Apply separately to the plate as bands, 20 mm by 3 mm, 10 µl of each of the following solutions: for solution A, add to 0.1 g of finely powdered test substance in a small test-tube, 0.05 ml of ammonia (~260 g/l) TS and 5 ml of chloroform R, stir vigorously with a glass rod, allow to stand for 30 minutes and filter; for solution B, dilute 1 ml of solution A to 25 ml with chloroform R; for solution C, dissolve 5 mg of emetine hydrochloride RS and 6 mg of cephaëline hydrochloride R in sufficient methanol R to produce 20 ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the odour of solvent is no longer detectable, spray it with a mixture of 0.05 g of iodine R in 10 ml of chloroform R, and heat it at 60 °C for 10 minutes. Examine the chromatogram first in daylight; a lemon-yellow zone appears at about midpoint, corresponding to emetine, and below it a light brown zone, corresponding to cephaëline. Then examine the chromatogram in ultraviolet light (365 nm); the zone corresponding to emetine shows an intense yellow fluorescence, and that corresponding to cephaëline, a light blue fluorescence. The chromatogram obtained with solution A shows, in addition, several very small zones due to secondary alkaloids. The chromatogram obtained with solution B shows only 2 zones, which correspond to those obtained with solution C.

With *Cephaëlis ipecacuanha*, the zone corresponding to cephaëline obtained with solution A is much smaller than the corresponding zone obtained with solution C.

With *Cephaëlis acuminata*, the principal zones obtained with solution A correspond in position, appearance, and intensity with those obtained with solution C.

Ash. Carry out the procedure as described under 4.1 Determination of ash and acid-insoluble ash; not more than 60 mg/g.

Acid-insoluble ash. Carry out the procedure as described under 4.1 Determination of ash and acid-insoluble ash; not more than 30 mg/g.

Foreign matter. Weigh about 200 g and spread it in a thin layer on a glass plate. Detect the foreign matter by eye or with the use of a 6× lens, separate it from the root, and weigh it; not more than 10 mg/g.

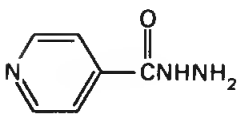
Assay. Weigh accurately about 7.5 g of finely powdered test substance, transfer it to a dry flask, add 100 ml of ether R, and shake for 5 minutes. Add 5 ml of ammonia (~100 g/l) TS, shake frequently during 1 hour, add 5 ml of water, and shake vigorously; decant the ether layer into a dry flask, filtering through a plug of adsorbent cotton. Wash the residue with two quantities, each of 25 ml of ether R, decanting each portion and filtering through the same plug of adsorbent cotton. Remove most of the solvent from the combined ether extracts by distillation and the remainder by gentle warming with a current of air blown into the flask. Dissolve the residue in 5 ml of previously neutralized ethanol (~710 g/l) TS by warming on a water-bath, add 15 ml of hydrochloric acid (0.1 mol/l) VS and titrate the excess of acid with sodium hydroxide (0.1 mol/l) VS, using 0.5 ml of methyl red/methylthioninium chloride TS as indicator. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 24.03 mg of total alkaloids, calculated as emetine.

ISONIAZIDUM
ISONIAZID

Molecular formula. $C_6H_7N_3O$

Relative molecular mass. 137.1

Graphic formula.



Chemical name. 4-Pyridinecarboxylic acid hydrazide; CAS Reg. No. 54-85-3.

Other name. Isonicotinic acid hydrazide.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 8 parts of water and in 40 parts of ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Tuberculostatic.

Storage. Isoniazid should be kept in a well-closed container, protected from light.

Requirements

Definition. Isoniazid contains not less than 98.0% and not more than 101.0% of $C_6H_7N_3O$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS or with the *reference spectrum* of isoniazid.

B. Heat 0.05 g with about 1 g of anhydrous sodium carbonate R; pyridine, perceptible by its odour, is produced.

C. Dissolve 0.1 g in 2 ml of water and add 10 ml of a hot solution of vanillin (10 g/l) TS, scratch the inside of the test-tube and allow to stand; a yellow precipitate is obtained. Filter, recrystallize from 5 ml of ethanol (~600 g/l) TS, and dry at 105 °C; melting temperature, about 227 °C.

Melting range. 170–174 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 6.0–8.0.

Free hydrazine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 98 volumes of acetone R and 2 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 1 volume of acetone R and 1 volume of water containing (A) 0.10 g of the test substance per ml, and (B) 20 µg of hydrazine hydrate R per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, spray with 4-dimethylaminobenzaldehyde TS3, and examine the chromatogram in daylight.

The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

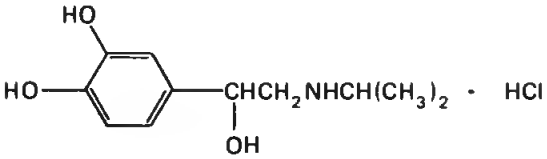
Assay. Dissolve about 0.25 g, accurately weighed, in sufficient water to produce 100 ml. To 25.0 ml of this solution add 100 ml of water, 20 ml of hydrochloric acid (~250 g/l) TS, 0.2 g of potassium bromide R, and 3 drops of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS, adding the titrant drop by drop and shaking till the red colour disappears. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 3.429 mg of $C_6H_7N_3O$.

ISOPRENALINI HYDROCHLORIDUM
ISOPRENALINE HYDROCHLORIDE

Molecular formula. $C_{11}H_{17}NO_3,HCl$

Relative molecular mass. 247.7

Graphic formula.



Chemical name. 3,4-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol hydrochloride; 4-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride; α-[(isopropylamino)methyl]protocatechuyl alcohol hydrochloride; CAS Reg. No. 51-30-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Bronchodilator.

Storage. Isoprenaline hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Isoprenaline hydrochloride gradually darkens in colour on exposure to air and light. Even in the absence of light, Isoprenaline

hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Isoprenaline hydrochloride contains not less than 97.5% and not more than 101.0% of $C_{11}H_{17}NO_3$,HCl, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 0.050 mg/ml solution, when observed between 240 nm and 350 nm, exhibits a maximum at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.50.

B. Add 1 ml of a 1.0 mg/ml solution to each of two flasks, one containing 10 ml of buffer phthalate, pH 3.4, TS, the other containing 10 ml of buffer phosphate, pH 6.4, TS; other buffers having the same pH may also be used. Add 0.5 ml of iodine (0.1 mol/l) VS, allow to stand for 5 minutes and add 2 ml of sodium thiosulfate (0.1 mol/l) VS. In the solution of pH 3.4, a strong red colour is produced; in the solution of pH 6.4, a strong red-violet colour is produced (distinction from levarterenol).

C. A 0.05 g/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of chlorides.

D. Melting temperature, about 169 °C with decomposition.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 4 hours; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5–6.0.

Isoprenalone. The absorbance of a 1-cm layer of a 1.0 mg/ml solution in sulfuric acid (0.005 mol/l) VS at 310 nm is not more than 0.2 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1 with the aid of the minimum of heat, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6

Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.77 mg of $C_{11}H_{17}NO_3$,HCl.

Additional requirements for Isoprenaline hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

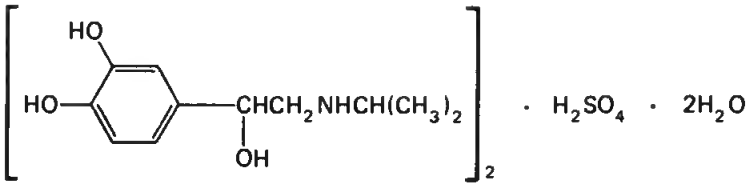
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1250.0 IU of endotoxin RS per mg.

ISOPRENALINI SULFAS
ISOPRENALINE SULFATE

Molecular formula. $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$

Relative molecular mass. 556.6

Graphic formula.



Chemical name. 3,4-Dihydroxy-α-[(isopropylamino)methyl]benzyl alcohol sulfate (2:1) (salt) dihydrate; 4-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol sulfate (2:1) (salt) dihydrate; α-[(isopropylamino)methyl]protocatechuyl alcohol sulfate (2:1) (salt) dihydrate: CAS Reg. No. 6700-39-6.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Bronchodilator.

Storage. Isoprenaline sulfate should be kept in a tightly closed container, protected from light.

Additional information. Isoprenaline sulfate gradually darkens in colour on exposure to air and light. Even in the absence of light, Isoprenaline sulfate is

gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Isoprenaline sulfate contains not less than 98.0% and not more than 101.0% of $(C_{11}H_{17}NO_3)_2,H_2SO_4$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 0.050 mg/ml solution, when observed between 240 nm and 350 nm, exhibits a maximum at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.50.

B. Add 1 ml of a 1.0 mg/ml solution to each of two flasks, one containing 10 ml of buffer phthalate, pH 3.4, TS, the other containing 10 ml of buffer phosphate, pH 6.4, TS; other buffers having the same pH may also be used. Add 0.5 ml of iodine (0.1 mol/l) VS, allow to stand for 5 minutes and add 2 ml of sodium thiosulfate (0.1 mol/l) VS. In the solution of pH 3.4, a strong red colour is produced; in the solution of pH 6.4, a strong red-violet colour is produced (distinction from levarterenol).

C. A 0.05 g/ml solution yields reaction A, described under 2.1 General identification tests as characteristic of sulfates.

D. Dissolve 0.1 g in 10 ml of ethanol (~750 g/l) TS, boil for 2 minutes and filter. Add 5 ml of ether R to the filtrate; a white, crystalline precipitate is formed; melting temperature, about 162 °C (anhydrous isoprenaline sulfate).

Clarity and colour of solution. A solution of 0.40 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.15 g of the substance; not less than 50 mg/g and not more than 75 mg/g.

pH value. pH of a 10 mg/ml solution, 4.0–5.5.

Isoprenalone. The absorbance of a 1-cm layer of a 1.0 mg/ml solution in sulfuric acid (0.005 mol/l) VS at 310 nm is not more than 0.2 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

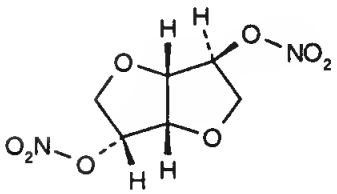
Assay. Dissolve about 0.45 g, accurately weighed, in 40 ml of glacial acetic acid R1 and 40 ml of acetonitrile R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 52.06 mg of $(C_{11}H_{17}NO_3)_2,H_2SO_4$.

Additional requirements for Isoprenaline sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1250.0 IU of endotoxin RS per mg.

ISOSORBIDI DINITRAS DILUTUS
DILUTED ISOSORBIDE DINITRATE



$C_6H_8N_2O_8$

CAUTION: To permit safe handling, Isosorbide dinitrate is mixed with a suitable inert diluent, such as lactose or mannitol.

Appropriate precautions in handling undiluted Isosorbide dinitrate need to be observed, since it can explode if subjected to percussion or excessive heat. Only exceedingly small amounts should be isolated.

Relative molecular mass. 236.1

Chemical name. 1,4: 3,6-Dianhydrosorbitol 2,5-dinitrate; CAS Reg. No. 87-33-2.

Description. Undiluted Isosorbide dinitrate is a fine, white, crystalline powder.

Solubility. Undiluted Isosorbide dinitrate is very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; very soluble in acetone R; freely soluble in dichloromethane R.

The solubility of the diluted product depends on the diluent and its concentration.

Category. Antianginal drug.

Storage. Diluted Isosorbide dinitrate should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container should state the percentage content of Isosorbide dinitrate, $C_6H_8N_2O_8$.

Additional information. Diluted Isosorbide dinitrate may contain a suitable stabilizer, such as up to 1% of ammonium phosphate.

Requirements

Diluted Isosorbide dinitrate contains not less than **95.0%** and not more than **105.0%** of the amount of $C_6H_8N_2O_8$ stated on the label. It usually contains 20–50% of Isosorbide dinitrate.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of isosorbide dinitrate. *(Instructions for the preparation of the spectrum will be given on the reference spectrum.)*

B. Dissolve in a test-tube a quantity equivalent to 10 mg of Isosorbide dinitrate in a mixture of 1.0 ml of water and about 2.0 ml of sulfuric acid (~1760 g/l) TS and cool. Introduce slowly 3.0 ml of ferrous sulfate (15 g/l) TS to form two layers; a brown colour is formed at the interface of the two liquids.

C. Shake a quantity equivalent to 25 mg of Isosorbide dinitrate with 10 ml of acetone R and filter. Evaporate the filtrate to dryness at a temperature not exceeding 40 °C and dry the residue under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 16 hours; melting point of the residue, about 71 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method B; not more than 10 µg/g.

Loss on drying. Dry at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 16 hours; it loses not more than 10 mg/g.

Inorganic nitrates. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 6 volumes of toluene R, 3 volumes of ethyl acetate R, and 1.5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 ml of each of the two following solutions. For solution (A) shake a quantity equivalent to 0.10 g of Isosorbide dinitrate in 5 ml of ethanol (~750 g/l) TS and filter. Solution (B) must be freshly prepared by dissolving 10 mg of potassium nitrate R in 1 ml of water and diluting to 100 ml with ethanol (~750 g/l) TS. After removing the plate from the chromatographic chamber, dry it in a current of air, and spray with freshly prepared potassium iodide/starch TS1. Expose the plate to ultraviolet light for 15 minutes. Examine the chromatogram in daylight.

Any spot corresponding to the nitrate ion obtained with solution A is not more intense than that obtained with solution B (0.5%, calculated as potassium nitrate).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 8 volumes of toluene R and 2 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 20 µl of each of the following 2 solutions. For solution (A) shake a quantity equivalent to 0.20 g of Isosorbide dinitrate with 5 ml of acetone R and filter. For solution (B) dilute 1 volume of solution A to 200 volumes with acetone R. After removing the plate from the chromatographic chamber, dry it in a current of air, and spray with diphenylamine/sulfuric acid TS. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%).

Assay. Shake a quantity equivalent to about 25 mg of Isosorbide dinitrate, accurately weighed, with 15 ml of glacial acetic acid R for 15 minutes. Dilute with sufficient glacial acetic acid R to produce 25 ml and filter. To 1.0 ml of the filtrate add 2 ml of phenoldisulfonic acid TS, allow to stand for 15 minutes, add 50 ml of water, make alkaline with ammonia (~260 g/l) TS, cool, and add sufficient water to produce 100 ml (solution A). Prepare similarly a solution containing 0.20 g of potassium nitrate R, previously dried at 105 °C, in 5 ml of water and add sufficient glacial acetic acid R to produce 25 ml. To 5 ml of the resulting solution add sufficient glacial acetic acid R to produce 50 ml. To 1.0 ml of this solution add 2 ml of phenoldisulfonic acid TS, allow to stand for 15 minutes, add 50 ml of water, make alkaline with ammonia (~260 g/l) TS, cool, and add sufficient water to produce 100 ml (solution B).

Measure the absorbance of a 1-cm layer at the maximum at about 405 nm of solution A against a solvent cell containing solution B, and calculate the content of $C_6H_8N_2O_8$.

Each ml of the potassium nitrate solution is equivalent to 0.934 mg of $C_6H_8N_2O_8$.

KALII CHLORIDUM
POTASSIUM CHLORIDE

Potassium chloride (non-injectable)
Potassium chloride for parenteral use

Molecular formula. KCl

Relative molecular mass. 74.55

Chemical name. Potassium chloride; CAS Reg. No. 7447-40-7.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 3 parts of water; practically insoluble in ethanol (~750 g/l) TS.

Category. Ionic equilibration agent.

Storage. Potassium chloride should be kept in a well-closed container.

Labelling. The designation Potassium chloride for parenteral use indicates that the substance complies with the additional requirement given at the end of this monograph and may be used for parenteral administration or for other sterile applications.

Requirements

Definition. Potassium chloride contains not less than 99.0% and not more than 100.5% of KCl, calculated with reference to the dried substance.

Identity tests

A. To a 0.05 g/ml solution add 2 ml of sodium hydroxide (~80 g/l) TS; it yields the reaction described under 2.1 General identification tests as characteristic of potassium.

B. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Iron. Use 1.0 g; the solution complies with the 2.2.4 Limit test for iron; not more than 40 µg/g.

Calcium and magnesium. To 20 ml of a 10 mg/ml solution add 2 ml of ammonia (~100 g/l) TS, 2 ml of ammonium oxalate (25 g/l) TS and 2 ml of disodium hydrogen phosphate (40 g/l) TS; no turbidity is produced within 5 minutes.

Barium. Dissolve 0.5 g in 10 ml of carbon-dioxide-free and ammonia-free water R and add 1 ml of sulfuric acid (~100 g/l) TS; no turbidity is produced within 1 minute.

Arsenic. Use a solution of 3.3 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Bromides. Dissolve 0.08 g in 10 ml of water, add 0.25 ml of sulfuric acid (0.5 mol/l) VS and 2 drops of tosylchloramide sodium (15 g/l) TS and mix; allow to stand for 2 minutes and extract with 5 ml of chloroform R. Prepare a reference solution by treating a mixture of 1.0 ml of potassium bromide (0.119 g/l) TS and 9 ml of water in a similar manner. Transfer the extracts to matched 10-ml stoppered test-tubes of transparent glass, add to each tube 3 ml of fuchsin TS, shake vigorously and allow to separate. The colour of the chloroform layer of the test solution when viewed transversely against a white background is not more intense than that produced in the reference solution when compared as described under 1.11 Colour of liquids.

Iodides. Moisten 5 g by adding, drop by drop, a recently prepared mixture composed of 25 ml of starch TS, 2 ml of sulfuric acid (0.5 mol/l) VS, 2 ml of sodium nitrite (10 g/l) TS, and 25 ml of water. Examine the mixture in daylight; no particles show any trace of blue colour within 5 minutes.

Sulfates. Dissolve 1.7 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.3 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Loss on drying. Dry to constant weight at 130 °C; it loses not more than 10 mg/g.

Acidity or alkalinity. Dissolve 5.0 g in 50 ml of carbon-dioxide-free water R, and add 0.1 ml of bromothymol blue/ethanol TS; not more than 0.2 ml of

sodium hydroxide (0.02 mol/l) VS or 0.2 ml of hydrochloric acid (0.02 mol/l) VS is required to obtain the midpoint of the indicator (green).

Assay. Dissolve about 1.0 g, accurately weighed, in sufficient water to produce 100 ml. To 10.0 ml add 50 ml of water, 5 ml of nitric acid (~130 g/l) TS, 25.0 ml of silver nitrate (0.1 mol/l) VS, and 2 ml of dibutyl phthalate R, shake the flask and titrate with ammonium thiocyanate (0.1 mol/l) VS using 2.5 ml of ferric ammonium sulfate (45 g/l) TS as indicator. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 7.455 mg of KCl.

Additional requirement for Potassium chloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 8.8IU of endotoxin RS per milliequivalence.

Sodium. Determine by atomic absorption spectrophotometry as described under 1.8 Atomic spectrometry: emission and absorption, at a wavelength of 589 nm; use a standard solution of sodium chloride R, previously dried to constant weight, dissolved in 1000 ml of water to contain 508.4 mg of NaCl (0.2 mg of Na per ml); the sodium content is not more than 1.0 mg/g.

Additional requirement for Potassium chloride for sterile use

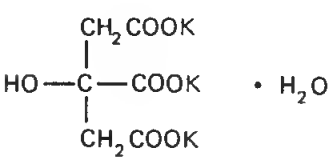
Complies with 3.2.1 Test for sterility of non-injectable preparations.

KALII CITRAS
POTASSIUM CITRATE

Molecular formula. $C_6H_5K_3O_7,H_2O$

Relative molecular mass. 324.4

Graphic formula.



Chemical name. Tripotassium citrate monohydrate; tripotassium 2-hydroxy-1,2,3-propanetricarboxylate monohydrate; CAS Reg. No. 6100-05-6 (mono-hydrate).

Description. Transparent crystals or a white, granular powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Systemic alkalinizing substance; component of oral rehydration salt mixtures.

Storage. Potassium citrate should be kept in a tightly closed container.

Additional information. Potassium citrate is deliquescent when exposed to moist air.

Requirements

Definition. Potassium citrate contains not less than 99.0% and not more than 101.0% of $C_6H_5K_3O_7$, calculated with reference to the anhydrous substance.

Identity tests

A. To a 0.1 g/ml solution add 2 ml of sodium hydroxide (~80 g/l) TS; it yields the reaction described under 2.1 General identification tests as characteristic of potassium.

B. A 0.1 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of citrates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sodium. Dissolve 1 g in 10 ml of water, add 6 ml of potassium antimonate TS, and allow to stand for 15 minutes; the solution is clear or any opalescence produced is not more pronounced than that of opalescence standard TS2.

Oxalates and tartrates. Dissolve 1 g in 4 ml of hydrochloric acid (~70 g/l) TS, add 4 ml of ethanol (~750 g/l) TS and 1.0 ml of calcium chloride (55 g/l) TS; the solution remains unchanged within 1 hour.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Readily carbonizable substances. Dissolve 0.20 g in 10 ml of sulfuric acid (~1760 g/l) TS and heat on a water-bath at 80–90 °C for 1 hour; the solution is not more intensely coloured than standard colour solutions Yw5 or Gn6 when compared as described under 1.11 Colour of liquids.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; stir and allow it to remain in contact with the dehydrated methanol R for 15 minutes, stir again for 1 minute, and then titrate; the water content is not less than 40 mg/g and not more than 70 mg/g.

Acidity or alkalinity. Dissolve 1 g in 10 ml of carbon-dioxide-free water R and add 0.1 ml of phenolphthalein/ethanol TS; not more than 0.2 ml of hydrochloric acid (0.1 mol/l) VS or 0.2 ml of sodium hydroxide (0.1 mol/l) VS is required to change the colour of the solution.

Assay. Dissolve about 0.15 g, accurately weighed, in 20 ml of glacial acetic acid R1, heat to about 50 °C, allow to cool to room temperature, add 0.25 ml of 1-naphtholbenzein/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS until a green colour is obtained as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 10.21 mg of $C_6H_5K_3O_7$.

KALII IODIDUM
POTASSIUM IODIDE

Molecular formula. KI

Relative molecular mass. 166.0

Chemical name. Potassium iodide; CAS Reg. No. 7681-11-0.

Description. Colourless crystals or a white, granular powder; odourless.

Solubility. Soluble in 0.7 part of water, in 17 parts of ethanol (~750 g/l) TS, in 2 parts of glycerol R, and in 75 parts of acetone R.

Category. Antifungal, expectorant.

Storage. Potassium iodide should be kept in a well-closed container, protected from light.

Requirements

Definition. Potassium iodide contains not less than 99.0% and not more than 101.0% of KI, calculated with reference to the dried substance.

Identity tests

A. To a 0.05 g/ml solution add 2 ml of sodium hydroxide (~80 g/l) TS; it yields the reaction described under 2.1 General identification tests as characteristic of potassium.

B. A 0.05 g/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of iodides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Barium. Dissolve 0.5 g in 10 ml of carbon-dioxide-free and ammonia-free water R and add 1 ml of sulfuric acid (~100 g/l) TS; no turbidity is produced within 1 minute.

Iodates. Dissolve 0.5 gin 10 ml of carbon-dioxide-free water R, and add 2 drops of sulfuric acid (~100 g/l) TS, followed by 1 drop of starch TS; there is no immediate production of a blue colour.

Nitrates, nitrites and ammonia. To a solution of 1.0 g in 5 ml of water contained in a test-tube of about 40 ml capacity, add 5 ml of sodium hydroxide (~80 g/l) TS and about 0.2 g of aluminium R wire. Insert a small plug of purified cotton wool in the upper part of the test-tube, and place a piece of moistened red litmus paper R over the mouth of the tube. Heat the test-tube with its contents in a water-bath for 15 minutes; no blue coloration of the paper is discernible.

Sulfates. Dissolve 2.5 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Thiosulfates. Dissolve 1.0 g in 10 ml of carbon-dioxide-free water R and add 0.1 ml of starch TS; not more than 0.05 ml of iodine (0.01 mol/l) VS is required to produce a blue colour.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Alkalinity. Dissolve 1.0 g in 10 ml of carbon-dioxide-free water R, add 0.1 ml of sulfuric acid (0.05 mol/l) VS, and 1 drop of phenolphthalein/ethanol TS; no colour is produced.

Assay. Dissolve about 0.3 g, accurately weighed, in 10 ml of water. Add 10 ml of a mixture of 50 ml of starch TS and 1 drop of iodine/ethanol TS. Titrate with silver nitrate (0.1 mol/l) VS until a pale yellow colour is produced. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 16.60 mg of KI.

KAOLINUM
KAOLIN

Chemical name. Kaolin; CAS Reg. No. 1332-58-7.

Other name. Bolus alba.

Description. A white or greyish white, unctuous powder, free from gritty particles; odour, clay-like.

Solubility. Practically insoluble in water and in organic solvents; insoluble in mineral acids and solutions of alkali hydroxides.

Category. Tablet and capsule diluent; suspending agent.

Storage. Kaolin should be kept in a well-closed container.

Labelling. The designation on the container of Kaolin should state whether it is intended for internal use.

Additional information. Attention should be paid to the microbiological quality, since Kaolin is of mineral origin.

Requirements

Definition. Kaolin is a purified, natural hydrated aluminium silicate, the composition of which is variable.

Identity tests

A. Boil 0.5 g with 1 g of sodium hydroxide R and 5 ml of water for 5 minutes. Dilute the mixture with sufficient water to produce 10 ml and filter. To 5 ml (keep the remaining filtrate for test B) add 0.5 g of ammonium chloride R, shake, and boil the solution; a white, gelatinous precipitate is produced.

B. To the filtrate from test A, add 1 ml of hydrochloric acid (~420 g/l) TS; an almost colourless, gelatinous precipitate is produced.

Acid-soluble substances. To 5 g add 7.5 ml of hydrochloric acid (~70 g/l) TS and 27.5 ml of water, and boil for 5 minutes. Filter, wash the residue with water, and dilute the combined filtrate and wash liquids with sufficient water to produce 50 ml. To 10 ml (keep the remaining solution for "Heavy metals") add 1.5 ml of sulfuric acid (~100 g/l) TS, evaporate to dryness on a water-bath, ignite, and weigh; the residue weighs not more than 10 mg (10 mg/g).

Heavy metals. To 5 ml of the solution prepared above add 5 ml of water, 10 ml of hydrochloric acid (~420 g/l) TS, and 25 ml of methylisobutylketone R. Shake for 2 minutes, allow to separate, and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of acetic acid (~300 g/l) TS and dilute to 40 ml with water. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 50 µg/g.

Iron. Triturate 2 g in a mortar with 10 ml of water and add 0.5 g of sodium salicylate R; not more than a slight red tint is produced.

Loss on ignition. Ignite to constant mass between 550 and 600 °C; it loses not more than 150 mg/g.

Acidity or alkalinity. To 1 g add 20 ml of carbon-dioxide-free water R, shake for 2 minutes, and filter. To 10 ml of the filtrate add 0.1 ml of phenolphthalein/ethanol TS; the solution remains colourless. Titrate with sodium hydroxide (0.01 mol/l) VS; not more than 0.25 ml is required to obtain a pink colour.

Swelling power. Triturate 2 g with 2 ml of water; the mixture does not flow.

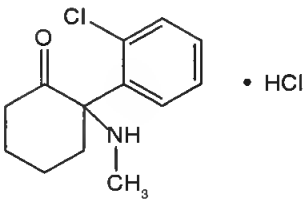
Adsorption capacity. Transfer 1 g to a 25-ml glass stoppered tube, add 10 ml of a solution containing 0.37 g of methylthioninium chloride R in 100 ml of water, and shake vigorously for 2 minutes. Centrifuge and dilute 1 ml of the supernatant solution to 100 ml with water; the colour produced is not darker than that of a solution containing 3.0 mg of methylthioninium chloride R in 100 ml of water.

Additional requirement for Kaolin intended for internal use

Heavy metals. To 10 ml of the solution prepared under "Acid-soluble substances", add 10 ml of water, 20 ml of hydrochloric acid (~420 g/l) TS, and 25 ml of methylisobutylketone R. Shake for 2 minutes, allow to separate, and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of acetic acid (~300 g/l) TS and dilute to 40 ml with water. Determine

the heavy metals content as described under 2.2.3 Limit test for heavy metals, Method A; not more than 25 µg/g.

KETAMINI HYDROCHLORIDUM
KETAMINE HYDROCHLORIDE



$C_{13}H_{16}ClNO,HCl$

Relative molecular mass. 274.2

Chemical name. (±)-2-(*o*-Chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride; (±)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride; CAS Reg. No. 1867-66-9.

Description. A white, crystalline powder; odour, characteristic.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. General anaesthetic.

Storage. Ketamine hydrochloride should be kept in a well-closed container.

Requirements

Ketamine hydrochloride contains not less than **98.5%** and not more than the equivalent of **101.0%** of $C_{13}H_{16}ClNO,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ketamine hydrochloride RS or with the *reference spectrum* of ketamine hydrochloride.

B. The absorption spectrum of a 0.33 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 269 nm and 276 nm. The ratio of the absorbance at 269 nm to that at 276 nm is between 1.10 and 1.22.

C. Dissolve 1 g in 10 ml of water and add 1 ml of sulfuric acid (~100 g/l) TS and 1 ml of ammonium reineckate (10 g/l) TS; a light red precipitate is produced.

D. A 0.1 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 258–261 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 2 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.1 g/ml solution, 3.5–4.1.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 49 volumes of cyclohexane R and 1 volume of isopropylamine R as the mobile phase. Apply separately to the plate 2 µl of each of two solutions in methanol R containing (A) 50 mg of Ketamine hydrochloride per ml and (B) 0.25 mg of Ketamine hydrochloride per ml. Develop the plate for a distance of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry in air and spray the plate evenly with modified Dragendorff reagent TS, dry, spray with hydrogen peroxide (~60 g/l) TS, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 1 ml of formic acid (~1080 g/l) TS and add 70 ml of a mixture of 6 volumes of acetic anhydride R and 1 volume of glacial acetic acid R1. Add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A.

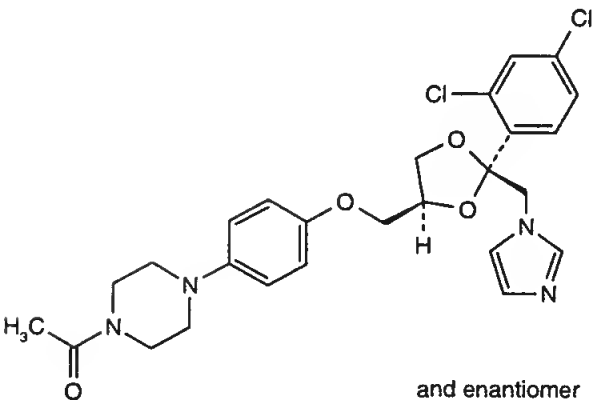
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.42 mg of $C_{13}H_{16}ClNO,HCl$.

Additional requirements for Ketamine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.4 IU of endotoxin RS per mg.

KETOCONAZOLUM
KETOCONAZOLE



$C_{26}H_{28}Cl_2N_4O_4$

Relative molecular mass. 531.4

Chemical name. ±-*cis*-1-Acetyl-4-[*p*-[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine; *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine; *cis*-1-acetyl-4-[*p*-[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-piperazine; CAS Reg. No. 65277-42-1.

Description. A white or almost white powder.

Solubility. Practically insoluble in water; freely soluble in dichloromethane R; soluble in methanol R; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antifungal drug.

Storage. Ketoconazole should be kept in a well-closed container, protected from light.

Requirements

Ketoconazole contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{26}H_{28}Cl_2N_4O_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ketoconazole RS or with the *reference spectrum* of ketoconazole.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Place 1 ml of nitric acid (~1000 g/l) TS in a porcelain dish and add 10 mg of the substance; a clear orange-red solution is produced.

D. Place 30 mg in a porcelain dish, add 0.3 g of anhydrous sodium carbonate R, and heat over an open flame for 10 minutes. Allow to cool, add 5 ml of nitric acid (~130 g/l) TS to the residue, stir, and filter. To 1 ml of the filtrate add 1 ml of water. The solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 148–152 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 4 volumes of dioxan R, 4 volumes of methanol R, and 2 volumes of ammonium acetate TS as the mobile phase. Apply separately to the plate 5 µl of each

of 5 solutions in the mobile phase containing (A) 6 mg of Ketoconazole per ml, (B) 6 mg of ketoconazole RS per ml, for (C) prepare a mixture of 6 mg of each of ketoconazole RS and econazole nitrate RS per ml, (D) 30 µg of ketoconazole RS per ml, and (E) 15 µg of ketoconazole RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air for 15 minutes. Expose the plate to iodine vapours until the spots appear and examine the chromatogram in daylight.

The test is not valid unless solution C shows two clearly separated spots. Any spot obtained with solution A, other than the principal spot, is not more intense than the principal spot obtained with solution D (0.5%) and only one such spot is more intense than that obtained with solution E (0.25%).

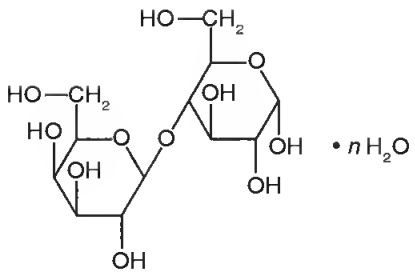
Assay. Dissolve about 0.2 g, accurately weighed, in 70 ml of a mixture of 1 volume of glacial acetic acid R1 and 7 volumes of ethylmethylketone R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.57 mg of $C_{26}H_{28}Cl_2N_4O_4$.

LACTOSUM

LACTOSE

Lactose, anhydrous
Lactose monohydrate



$n = 0$ (anhydrous)
$n = 1$ (monohydrate)

$C_{12}H_{22}O_{11}$ (anhydrous)
$C_{12}H_{22}O_{11},H_2O$ (monohydrate)

Relative molecular mass. 342.3 (anhydrous); 360.3 (monohydrate).

Chemical name. Lactose; 4-*O*-β-D-galactopyranosyl-D-glucose; CAS Reg. No. 63-42-3.

Lactose monohydrate; 4-*O*-β-D-galactopyranosyl-D-glucose monohydrate; CAS Reg. No. 64044-51-5.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Freely but slowly soluble in water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Tablet and capsule diluent.

Storage. Lactose should be kept in a well-closed container.

Labelling. The designation on the container of Lactose should state whether it is the monohydrate or the anhydrous form.

Additional information. Attention should be paid to the microbiological purity, since Lactose is of natural origin.

Requirements

Identity tests

A. Dissolve 0.1 g in 10 ml of water, add 3 ml of potassio-cupric tartrate TS, and heat; a red precipitate is formed.

B. Dissolve 0.25 g in 5 ml of water, add 5 ml of ammonia (~260 g/l) TS, and heat in a water-bath at 80 °C for 10 minutes; a red colour develops.

C. Dissolve 20 mg in 5 ml of water, add 0.2 ml of methylamine hydrochloride (20 g/l) TS, and heat to boiling for 30 seconds. Add 0.2 ml of sodium hydroxide (~200 g/l) TS; the initial yellow colour of the solution turns to red.

Specific optical rotation. Dissolve 10 g in 80 ml of water while heating to 50 °C. Allow to cool and add 0.2 ml of ammonia (~100 g/l) TS. After 30 minutes dilute to 100 ml with water. Measure the rotation at 20 °C and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +54.4$ to $+55.9$ °.

Heavy metals. Use 1.0 g for the preparation of the test solution, add 1 ml of hydrochloric acid (0.1 mol/l) VS and proceed as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 5 µg/g.

Starch. Dissolve 1.5 g in 10 ml of boiling water. Cool, and add 1 drop of iodine (0.05 mol/l) VS; no blue colour develops.

Clarity and colour of solution. A solution of 3 g in 10 ml of boiling water is colourless or nearly colourless, clear, and odourless.

Ethanol-soluble substances. Add 10 g of finely powdered Lactose to 40 ml of ethanol (~750 g/l) TS and shake for 10 minutes. Filter, evaporate 10 ml of the filtrate to dryness, dry the residue at 100 °C for 10 minutes, and weigh; not more than 20 mg.

Sulfated ash. Not more than 1.0 mg/g.

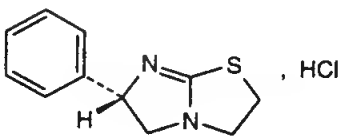
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using either:

— 2 g of anhydrous Lactose; the water content is not more than 10 mg/g; or

— 0.5 g of Lactose monohydrate; the water content is not less than 45 mg/g and not more than 55 mg/g.

Acidity or alkalinity. Boil 6 g with 25 ml of carbon-dioxide-free water R, cool, and add 0.3 ml of phenolphthalein/ethanol TS; the solution remains colourless. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS; not more than 0.4 ml is required to obtain the midpoint of the indicator (pink).

LEVAMISOLI HYDROCHLORIDUM
LEVAMISOLE HYDROCHLORIDE



$C_{11}H_{12}N_2S,HCl$

Relative molecular mass. 240.8

Chemical name. (-)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride; (*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride; CAS Reg. No. 16595-80-5.

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in dichloromethane R.

Category. Anthelminthic drug.

Storage. Levamisole hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Levamisole hydrochloride contains not less than **98.5%** and not more than **101.0%** of $C_{11}H_{12}N_2S,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levamisole hydrochloride RS or with the *reference spectrum* of levamisole hydrochloride.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution D.

C. Dissolve about 0.06g in 20ml of water, add 2ml of sodium hydroxide (~80g/l) TS, boil for 10 minutes, and cool. Add a few drops of sodium nitroprusside (45g/l) TS; a red colour is produced which fades on standing.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 0.050g/ml solution in carbon-dioxide-free water R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = 121.5°$ to $-128°$.

Clarity and colour of solution. A solution of 0.50g in 10ml of carbondioxide- free water R is clear and not more intensely coloured than standard colour Yw1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0mg/g.

Loss on drying. Dry to constant mass at 105°C; it loses not more than 5.0mg/g.

pH value. pH of a 0.05 g/ml solution, 3.5–5.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 60 volumes of toluene R, 40 volumes of acetone R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in methanol R containing (A) 50 mg of Levamisole hydrochloride per ml, (B) 5.0 mg of Levamisole hydrochloride per ml, (C) 0.25 mg of Levamisole hydrochloride per ml, and (D) 5.0 mg of levamisole hydrochloride RS per ml. After removing the plate from the chromatographic chamber, dry it at 105 °C for 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

Expose the plate to iodine vapour in a tightly closed chamber for 15 minutes and examine the chromatogram in daylight.

Any spot obtained with solution A, other than any spot with a very low *Rf* value, is not more intense than that obtained with solution C (0.5%).

Assay. Dissolve about 0.2 g, accurately weighed, in 30 ml of ethanol (~750 g/l) TS and add 5 ml of hydrochloric acid (0.01 mol/l) VS. Titrate with sodium hydroxide (0.1 mol/l) VS, determining the two inflection points potentiometrically. Record the volume, in ml, of sodium hydroxide (0.1 mol/l) VS consumed between the two inflection points.

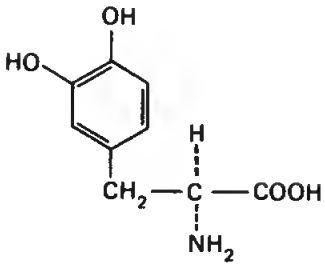
Each ml of sodium hydroxide (0.1mol/l) VS is equivalent to 24.08 mg of $C_{11}H_{12}N_2S,HCl$.

LEVODOPUM
LEVODOPA

Molecular formula. $C_9H_{11}NO_4$

Relative molecular mass. 197.2

Graphic formula.



Chemical name. (-)-3-(3,4-Dihydroxyphenyl)-L-alanine; 3-hydroxy-L-tyrosine; CAS Reg. No. 59-92-7.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 300 parts of water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Antiparkinsonism drug.

Storage. Levodopa should be kept in a tightly closed container, protected from light, and stored in a cool place.

Requirements

Definition. Levodopa contains not less than 98.5% and not more than 101.0% of $C_9H_{11}NO_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levodopa RS or with the *reference spectrum* of levodopa.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. To 5 mg add 1 ml of water, 1 ml of pyridine R and 5 mg of 4-nitrobenzoyl chloride R, mix and allow to stand for 3 minutes; a violet colour is produced which changes to pale yellow on boiling. While shaking, add 0.1 ml of sodium carbonate (200 g/l) TS; the violet colour reappears.

Specific optical rotation. Transfer about 0.5 g, accurately weighed, to a 25-ml volumetric flask, dissolve it in 10 ml of hydrochloric acid (1 mol/l) VS, add 5 g of methenamine R, swirl the contents to dissolve the methenamine, dilute with sufficient hydrochloric acid (1 mol/l) VS to produce 25 ml, mix, and allow to stand in the dark at 25 °C for 3 hours; $[\alpha]_D^{20\,°C} = -160$ to -167 °.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Clarity and colour of solution. Dissolve 0.50 g in a mixture of 2 ml of hydrochloric acid (~70 g/l) TS and 8 ml of water; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R2 as the coating substance and a mixture of 50 volumes of 1-butanol R, 25 volumes of glacial acetic acid R, and 25 volumes of water as the mobile phase. Prepare a fresh solution of 0.10 g of the test substance dissolved in 5 ml of anhydrous formic acid R and dilute to 10 ml with methanol R; this constitutes solution A. Dilute 0.5 ml of solution A to 100 ml with methanol R; this constitutes solution B. Separately prepare a fresh solution of 0.10 g of levodopa RS dissolved in 5 ml of anhydrous formic acid R and dilute to 10 ml with methanol R. Dilute 0.5 ml of this solution to 100 ml with methanol R; this constitutes solution C. Dissolve 30 mg of tyrosine R in 1 ml of anhydrous formic acid R and dilute to 100 ml with methanol R. Mix 1 ml of this solution with 1 ml of solution A; this constitutes solution D. Apply separately to the plate 10 µl of each of solutions A, B, and C, and 20 µl of solution D. Dry the plate in a stream of air before placing it in the chromatographic chamber. Develop the plate, remove it from the chamber, allow it to dry in air, spray with a freshly prepared solution containing 2 volumes of ferric chloride (25 g/l) TS and 1 volume of potassium ferricyanide (50 g/l) TS, and examine the chromatogram immediately in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots.

Assay. Dissolve by heating about 0.18 g, accurately weighed, in 5 ml of anhydrous formic acid R, add 25 ml of glacial acetic acid R1 and 25 ml of dioxan R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-

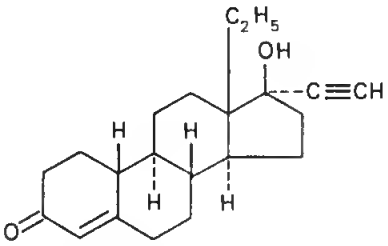
aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 19.72 mg of $C_9H_{11}NO_4$.

LEVONORGESTRELUM
LEVONORGESTREL

Molecular formula. $C_{21}H_{28}O_2$

Relative molecular mass. 312.5

Graphic formula.



Chemical name. (–)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one; CAS Reg. No. 797-63-7.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and ether R.

Category. Contraceptive.

Storage. Levonorgestrel should be kept in a well-closed container, protected from light.

Requirements

Definition. Levonorgestrel contains not less than 98.0% and not more than 102.0% of $C_{21}H_{28}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the

spectrum obtained from levonorgestrel RS or with the *reference spectrum* of levonorgestrel.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Melting temperature, about 236 °C.

Specific optical rotation. Use a 10 mg/ml solution in chloroform R; $[\alpha]_D^{20\,°C}$ = –30.0 to –35.0 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity or alkalinity. Dissolve 0.10 g in 30 ml of dehydrated ethanol R and add 0.5 ml of methyl red/ethanol TS; not more than 0.15 ml of sodium hydroxide (0.01 mol/l) VS is required to obtain a yellow colour and not more than 0.30 ml of hydrochloric acid (0.01 mol/l) VS is required to obtain a red colour.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 8 volumes of chloroform R and 2 volumes of acetone R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in chloroform R containing (A) 10 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of levonorgestrel RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray it with a mixture of 50 ml of methanol R and 10 ml of sulfuric acid (~1760 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Ethynyl group. Dissolve about 0.2 g, accurately weighed, in about 40 ml of tetrahydrofuran R. Add 10 ml of silver nitrate (100 g/l) TS and titrate with sodium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically, using a glass and a calomel electrode that contains potassium nitrate solution as the electrolyte. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 2.503 mg of –C≡CH; the content of ethynyl group is not less than 78.1 mg per g and not more than 81.8 mg per g.

Assay. Dissolve about 0.05 g, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 2.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum

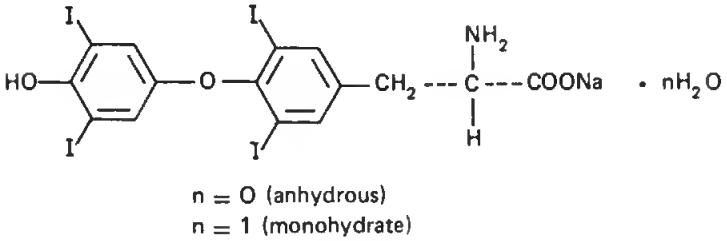
at about 241 nm. Calculate the amount of $C_{21}H_{28}O_2$ in the substance being tested by comparison with levonorgestrel RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.54 ± 0.03.

LEVOTHYROXINUM NATRICUM
LEVOTHYROXINE SODIUM

Molecular formula. $C_{15}H_{10}I_4NNaO_4$ (anhydrous); $C_{15}H_{10}I_4NNaO_4,H_2O$ (mono-hydrate).

Relative molecular mass. 798.9 (anhydrous); 816.9 (monohydrate).

Graphic formula.



Chemical name. Monosodium L-thyroxine; monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine; 3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-L-alanine monosodium salt; CAS Reg. No. 55-03-8 (anhydrous); Monosodium L-thyroxine monohydrate; monosodium *O*-(4-hydroxy-3,5-diiodo-phenyl)-3,5-diiodo-L-tyrosine monohydrate; 3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-L-alanine monosodium salt monohydrate; CAS Reg. No. 31178-59-3 (monohydrate).

Other name. Thyroxine sodium.

Description. An almost white or slightly coloured powder, or a fine, slightly coloured, crystalline powder; odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R. It dissolves in solutions of alkali hydroxides.

Category. Thyroid hormone.

Storage. Levothyroxine sodium should be kept in a tightly closed container, protected from light.

Additional information. Levothyroxine sodium may contain a variable quantity of water of crystallization; anhydrous levothyroxine sodium is hygroscopic.

Requirements

Definition. Levothyroxine sodium contains not less than 97.0% and not more than 101.0% of $C_{15}H_{10}I_4NNaO_4$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 2.0 ml of nitric acid (~130 g/l) TS and warm the solution; a brown to violet colour is produced. Cool, add 1.0 ml of chloroform R and shake; the colour of the chloroform layer turns violet.

B. Dissolve 5 mg in a mixture of 2.0 ml of ethanol (~750 g/l) TS and about 0.2 ml of hydrochloric acid (~70 g/l) TS, add about 0.25 ml of sodium nitrite (10 g/l) TS and allow to stand for 15 minutes or heat for 2–3 minutes in a water-bath; a yellow solution is produced. Cool and add ammonia (~100 g/l) TS to make the solution alkaline; the colour changes to red.

C. Examine the chromatograms obtained in the test for liothyronine (see below). The principal spot in the chromatogram obtained with solution A is similar in position, colour, and size to the principal spot in the chromatogram obtained with reference solution B.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, ignite 20 mg and dissolve the residue in acetic acid (~60 g/l) TS.

Specific optical rotation. Dissolve 0.5 g in 22 ml of a gently boiling mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 4 volumes of ethanol (~750 g/l) TS. Cool and dilute to 25 ml with the same mixture of solvents. Calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +16.0 to +20.0 °C.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 60 mg/g and not more than 120 mg/g.

Liothyronine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate coated with a mixture of 30 g of silica gel R3 and 60 ml of a solution containing 0.75 g of soluble starch R in 100 ml of water. Use the plate directly without heating. As the mobile phase use a mixture of 20 volumes of ammonia (~260 g/l) TS, 35 volumes of 2-propanol R, and 55 volumes

of ethyl acetate R. For the preparation of the test and reference solutions, make up the following solvent mixture: to 5 volumes of ammonia (~260 g/l) TS add 70 volumes of methanol R and mix. For solution A, dissolve 0.10 g of the test substance in the solvent mixture to produce 5 ml of concentrated solution, then dilute 1.0 ml of this solution with the same solvent mixture to produce 2.0 ml of diluted solution. For solution B, dissolve 50 mg of levothyroxine sodium RS in the ammonia/methanol solvent mixture to produce 5 ml. For solution C, dissolve 5 mg of liothyronine RS in the ammonia/methanol solvent mixture to produce 25 ml of concentrated solution, then dilute 1.0 ml of this solution with the same solvent mixture to produce 2.0 ml of diluted solution. For solution D, mix 1.0 ml of the concentrated solution A with 1.0 ml of the concentrated solution C. Apply separately to the plate 5 µl of each of diluted solution A, solution B, diluted solution C, and solution D. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with ferric chloride/ferricyanide/arsenite TS, and examine the chromatogram in daylight. Any spot corresponding to liothyronine in the chromatogram obtained with solution A is not more intense than the spot in the chromatogram obtained with solution C. The test is not valid unless the chromatogram obtained with solution D shows two clearly separated spots.

Soluble halides. Shake 10 mg with 10 ml of water containing about 0.05 ml of nitric acid (~130 g/l) TS for 5 minutes and filter. Dilute the filtrate to 10 ml with water and add 0.15 ml of silver nitrate (40 g/l) TS; any opalescence produced is not more intense than that of a solution simultaneously prepared by adding 0.15 ml of silver nitrate (40 g/l) TS and 0.10 ml of hydrochloric acid (0.02 mol/l) VS to 10 ml of water (7 mg/g as chlorides).

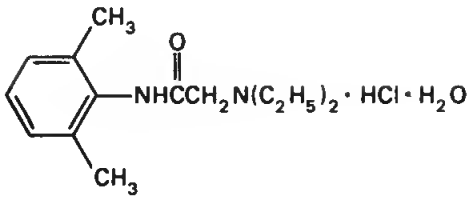
Assay. Carry out the combustion as described under 2.4 Oxygen flask method, using about 25 mg of the test substance, accurately weighed, and 10 ml of sodium hydroxide (10 g/l) TS as the absorbing liquid. When the process is complete, proceed as described under the 4.2 Determination of iodine value. Each ml of sodium thiosulfate (0.05 mol/l) VS is equivalent to 1.665 mg of $C_{15}H_{10}I_4NNaO_4$.

LIDOCAINI HYDROCHLORIDUM
LIDOCAINE HYDROCHLORIDE

Molecular formula. $C_{14}H_{22}N_2O,HCl,H_2O$

Relative molecular mass. 288.8

Graphic formula.



Chemical name. 2-(Diethylamino)-2′,6′ acetoxylidide monohydrochloride monohydrate; 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide monohydrochloride monohydrate; CAS Reg. No. 6108-05-0.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 0.7 part of water and in 1.5 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Local anaesthetic.

Storage. Lidocaine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Lidocaine hydrochloride causes local numbness after being placed on the tongue. Even in the absence of light, Lidocaine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Lidocaine hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{14}H_{22}N_2O$,HCl, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and C or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of lidocaine hydrochloride.

B. Dissolve 0.15 g in 10 ml of water, make the solution alkaline with sodium hydroxide (~80 g/l) TS, filter, and wash the precipitate with water. Dissolve the precipitate in 1 ml of ethanol (~750 g/l) TS, add 0.5 ml of cobaltous chloride TS, and shake for 2 minutes; a bluish green precipitate is produced.

C. A 0.05 g/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of chlorides.

D. Dissolve 0.1 g in 10 ml of water and add 10 ml of trinitrophenol (7 g/l) TS. Filter, wash the precipitate with water and dry at 105 °C. Melting temperature, about 230 °C (picrate).

Melting range. 74–79 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 50 mg/g and not more than 75 mg/g.

pH value. pH of a 0.05 g/ml solution, 4.0–5.5.

Primary aromatic amines. Dissolve 0.10 g in 4 ml of hydrochloric acid (~70 g/l) TS using a 100-ml volumetric flask. Cool the solution in an ice-bath. In a test-tube, dissolve 50 mg of sodium nitrite R in 10 ml of water and cool the solution. To the flask in the ice-bath add half of the volume of the cooled sodium nitrite solution. Allow to stand for 10 minutes, lift the flask from the ice-bath, and add 1 g of urea R. Shake the flask frequently and when the evolution of gas has ceased (about 15 minutes), add 2.5 ml of sodium hydroxide (~80 g/l) TS in which 10 mg of thymol R has previously been dissolved. Add 5 ml of sodium hydroxide (~80 g/l) TS, allow to stand for 10 minutes, and dilute to volume. Prepare a blank as described above, but without the substance being examined. The test solution is not more intensely coloured than the blank solution when compared as described under 1.11 Colour of liquids.

Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.08 mg of $C_{14}H_{22}N_2O,HCl$.

Additional requirements for Lidocaine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

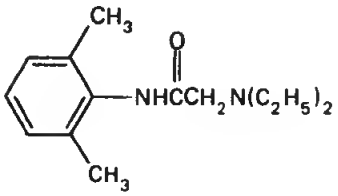
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.1 IU of endotoxin RS per mg.

LIDOCAINUM
LIDOCAINE

Molecular formula. $C_{14}H_{22}N_2O$

Relative molecular mass. 234.3

Graphic formula.



Chemical name. 2-(Diethylamino)-2',6'-acetoxylidide; 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide; CAS Reg. No. 137-58-6.

Description. A white or slightly yellow, crystalline powder; odour, characteristic.

Solubility. Practically insoluble in water; very soluble in ethanol (~750 g/l) TS; freely soluble in benzene R and ether R.

Category. Local anaesthetic.

Storage. Lidocaine should be kept in a tightly closed container, protected from light.

Additional information. Lidocaine causes local numbness after being placed on the tongue.

Requirements

Definition. Lidocaine contains not less than 99.0% and not more than 101.0% of $C_{14}H_{22}N_2O$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from lidocaine RS or with the *reference spectrum* of lidocaine.

B. Dissolve 0.1 g in 1 ml of ethanol (~750 g/l) TS, add 0.5 ml of cobaltous chloride TS, and shake for 2 minutes; a bluish green precipitate is produced.

C. Dissolve 0.1 g in 15 ml of ethanol (~750 g/l) TS and add 10 ml of trinitrophenol (7 g/l) TS. Filter, wash the precipitate with water, and dry at 105 °C. Melting temperature, about 230 °C (picrate).

Melting range. 66–69 °C.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in a mixture of 4 ml of hydrochloric acid (~70 g/l) TS and 21 ml of water, and proceed as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 0.50 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, filter if necessary, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.5 mg/g.

Sulfates. Dissolve 0.50 g in 5 ml of hydrochloric acid (~70 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight over phosphorus pentoxide R at ambient temperature; it loses not more than 5.0 mg/g.

Primary aromatic amines. Dissolve 0.10 g in 4 ml of hydrochloric acid (~70 g/l) TS using a 100-ml volumetric flask. Cool the solution in an ice-bath. In a test-tube, dissolve 50 mg of sodium nitrite R in 10 ml of water and cool the solution. To the flask in the ice-bath, add half of the volume of the cooled sodium nitrite solution. Allow to stand for 10 minutes, lift the flask from the ice-bath, and add 1 g of urea R. Shake the flask frequently and when the evolution of gas has ceased (about 15 minutes), add 2.5 ml of sodium hydroxide (~80 g/l) TS in which 10 mg of thymol R have previously been dissolved. Add 5 ml of sodium hydroxide (~80 g/l) TS, allow to stand for 10 minutes and dilute to volume. Prepare a blank as described above, but without the substance being examined. The test solution is not more intensely coloured than the blank solution when compared as described under 1.11 Colour of liquids.

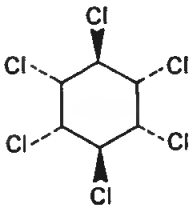
Assay. Dissolve about 0.45 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 23.43 mg of $C_{14}H_{22}N_2O$.

LINDANUM
LINDANE

Molecular formula. $C_6H_6Cl_6$

Relative molecular mass. 290.8

Graphic formula.



Chemical name. γ-1,2,3,4,5,6-Hexachlorocyclohexane; (1α,2α,3β,4α,5α,6β)-1,2,3,4,5,6-hexachlorocyclohexane; CAS Reg. No. 58-89-9.

Other names. Gamma benzene hexachloride; gammahexachlorcyclohexane.

Description. A white, crystalline powder; odour, slight.

Solubility. Practically insoluble in water; soluble in dehydrated ethanol R and ether R.

Category. Pediculicide; scabicide.

Storage. Lindane should be kept in a well-closed container.

Requirements

Definition. Lindane contains not less than 99.0% and not more than 100.5% of $C_6H_6Cl_6$, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from lindane RS or with the *reference spectrum* of lindane.

B. To 1 ml of a 5.0 mg/ml solution add 3 ml of ethanol (~750 g/l) TS and 1 ml of potassium hydroxide/ethanol TS 1 and allow to stand for 10 minutes; the mixture yields reaction B, described under 2.1 General identification tests as characteristic of chlorides.

Congealing temperature. Not below 112.0 °C.

Free chlorides. For the preparation of the test solution shake 1.2 g with 30 ml of water for 1 minute, and filter. To the filtrate add 10 ml of nitric acid (~130 g/l) TS and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 5.0 mg/g.

Acidity or alkalinity. Boil 1.5 g with 30 ml of water for 1 minute and filter. To 10 ml of the filtrate add 2 drops of phenolphthalein/ethanol TS and titrate with carbonate-free sodium hydroxide (0.01 mol/l) VS; not more than 0.2 ml is required to obtain a pink colour. Add 0.4 ml of hydrochloric acid (0.01 mol/l) VS and 5 drops of methyl red/ethanol TS; the colour changes to orange.

Assay. To about 0.4 g, accurately weighed, add 25 ml of ethanol (~750 g/l) TS and warm on a water-bath until dissolved. Cool, add 10 ml of potassium hydroxide/ethanol (1 mol/l) VS, swirl gently, and allow to stand for 10 minutes. Dilute to 150 ml with water, neutralize with nitric acid (~130 g/l) TS, add 10 ml in excess of the acid, followed by 50 ml of silver nitrate (0.1 mol/l) VS. Filter, wash the residue with water, and titrate the combined filtrate and washings with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 9.693 mg of $C_6H_6Cl_6$.

LITHII CARBONAS
LITHIUM CARBONATE

Molecular formula. Li_2CO_3

Relative molecular mass. 73.89

Chemical name. Dilithium carbonate; CAS Reg. No. 554-13-2.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in 100 parts of water; less soluble in boiling water; very slightly soluble in ethanol (~750 g/l) TS.

Category. Antidepressant.

Storage. Lithium carbonate should be kept in a well-closed container.

Additional information. Lithium carbonate has a slightly alkaline taste.

Requirements

Definition. Lithium carbonate contains not less than 99.5% and not more than 100.5% of Li_2CO_3, calculated with reference to the dried substance.

Identity tests

A. Moisten a few crystals with hydrochloric acid (~420 g/l) TS and introduce them on a platinum wire into the flame of a Bunsen burner; a carmine-red colour is produced in the flame.

B. Dissolve 0.2 g in 5 ml of hydrochloric acid (~420 g/l) TS, boil, add 2 ml of sodium hydroxide (~80 g/l) TS, 5 ml of disodium hydrogen phosphate (40 g/l) TS and boil; a white precipitate is produced.

C. To a small amount add hydrochloric acid (~70 g/l) TS; it effervesces and the gas is colourless. Add a few drops of calcium hydroxide TS; immediately a white precipitate is formed.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in 10 ml of acetic acid (~60 g/l) TS, adjust the pH to 3–4, dilute to 40 ml with water and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Arsenic. Use a solution of 5.0 g dissolved in a mixture of 15 ml of brominated hydrochloric acid AsTS and 45 ml of water and remove the excess bromine with a few drops of stannous chloride AsTS; proceed with the solution as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Calcium and magnesium. Dissolve 1.0 g in 30 ml of hydrochloric acid (1 mol/l) VS and neutralize with ammonia (~100 g/l) TS, filter if necessary, and divide into 2 equal portions. To one portion add 1 ml of ammonium oxalate (25 g/l) TS; no turbidity or precipitate is produced when the mixture is allowed to stand for 5 minutes (limit of calcium). To the second portion add 1 ml of disodium hydrogen phosphate (40 g/l) TS; no turbidity or precipitate is produced when the mixture is allowed to stand for 5 minutes (limit of magnesium).

Chlorides. Dissolve 0.35 gin a mixture of 3 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.7 mg/g.

Sodium. Determine by atomic absorption spectrophotometry "1.8 Atomic spectrometry: emission and absorption" at a wavelength of 589 nm; use a standard solution prepared by dissolving sodium chloride R, previously dried to constant weight, in 1000 ml of water to give a solution containing 508.4 mg of NaCl (0.2 mg of Na per ml); the sodium content is not more than 2.0 mg/g.

Sulfates. Dissolve 0.5 g in 20 ml of water, add 3 ml of hydrochloric acid (~250 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

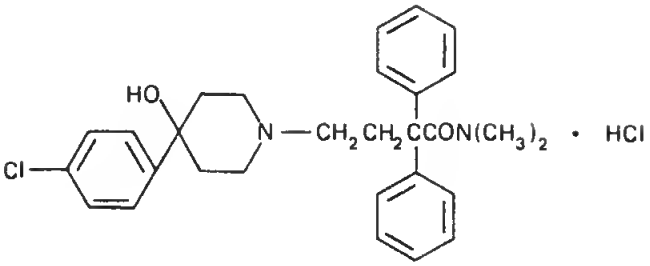
Assay. Dissolve about 0.75 g, accurately weighed, in 100 ml of water, add 50 ml of hydrochloric acid (1 mol/l) VS, boil to remove carbon dioxide, cool, and titrate the excess acid with sodium hydroxide (1 mol/l) VS, using methyl orange/ethanol TS as indicator. Each ml of hydrochloric acid (1 mol/l) VS is equivalent to 36.95 mg of Li_2CO_3.

LOPERAMIDI HYDROCHLORIDUM
LOPERAMIDE HYDROCHLORIDE

Molecular formula. $C_{29}H_{33}ClN_2O_2,HCl$

Relative molecular mass. 513.5

Graphic formula.



Chemical name. 4-(*p*-Chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide monohydrochloride; 4-(4-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutanamide monohydrochloride; CAS Reg. No. 34552-83-5.

Description. A white to slightly yellowish powder.

Solubility. Slightly soluble in water and in dilute acids; freely soluble in methanol R.

Category. Antidiarrhoeal drug.

Storage. Loperamide hydrochloride should be kept in a well-closed container.

Requirements

Definition. Loperamide hydrochloride contains not less than 98.0% and not more than 102.0% of $C_{29}H_{33}ClN_2O_2$,HCl, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from loperamide hydrochloride RS or with the *reference spectrum* of loperamide hydrochloride.

B. Transfer about 0.04 g, accurately weighed, to a 100-ml volumetric flask, dissolve in about 50 ml of 2-propanol R, add 10 ml of hydrochloric acid (0.1 mol/l) VS and dilute to volume with 2-propanol R. The absorption spectrum of this solution against a solvent cell containing the same solvent mixture, when observed between 230 nm and 350 nm, is qualitatively similar to that of a solution of loperamide hydrochloride RS concurrently examined (maxima occur at about 253 nm, 259 nm, 265 nm, and 273 nm).

The absorbances of the solutions at the respective maxima do not differ from each other by more than 3%.

C. Melting temperature, about 224 °C with decomposition.

D. A 10 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 85 volumes of chloroform R, 10 volumes of methanol R, and 5 volumes of formic acid (~1080 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 10 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and expose it to iodine vapours. Examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.38 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 51.35 mg of $C_{29}H_{33}ClN_2O_2$,HCl.

MAGNESII HYDROXIDUM
MAGNESIUM HYDROXIDE

Molecular formula. $Mg(OH)_2$

Relative molecular mass. 58.32

Chemical name. Magnesium hydroxide; CAS Reg. No. 1309-42-8.

Description. A white, fine, amorphous powder; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in dilute acids.

Category. Antacid.

Storage. Magnesium hydroxide should be kept in a tightly closed container.

Requirements

Definition. Magnesium hydroxide contains not less than 95.0% and not more than 100.5% of $Mg(OH)_2$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1.0 ml of hydrochloric acid (~70 g/l) TS, add 1.0 ml of ammonium chloride (100 g/l) TS, 0.5 ml of disodium hydrogen phosphate (100 g/l) TS, and 1.0 ml of ammonia (~100 g/l) TS; a white, crystalline precipitate is formed, which is soluble in acetic acid (~300 g/l) TS.

B. Dissolve 10 mg in 1.0 ml of hydrochloric acid (~70 g/l) TS, and add 2.0 ml of sodium hydroxide (~80 g/l) TS; a white, gelatinous precipitate is produced, which is insoluble in an excess of sodium hydroxide (~80 g/l) TS. Add a few drops of iodine TS; the precipitate turns dark brown.

Heavy metals. Dissolve 1.0 g in 15 ml of hydrochloric acid (~250 g/l) TS and shake with 25 ml of methylisobutylketone R for 2 minutes. Allow to stand, separate the layers, and evaporate the aqueous layer to dryness. Dissolve the residue in 15 ml of water and proceed as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 30 µg/g.

Arsenic. Use a solution of 3.3 g in 20 ml of sulfuric acid (~100 g/l) TS and 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Calcium. Dissolve 5.0 g in a mixture of 50 ml of acetic acid (~300 g/l) TS and 50 ml of water, boil for 2 minutes, cool, and dilute to 100 ml with acetic acid (~120 g/l) TS. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. Dilute 1.3 ml of the filtrate to 150 ml with water (retain the filter for the test of substances insoluble in acetic acid). To 0.20 ml of ethanolic calcium standard (100 µg/ml Ca) TS add 0.8 ml of ammonium oxalate (50 g/l) TS. After 1 minute add 1 ml of acetic acid (~120 g/l) TS and 15 ml of the diluted filtrate prepared above.

Prepare similarly a standard solution using a mixture of 10 ml of calcium standard (10 µg/ml Ca) TS and 5 ml of water.

After 15 minutes any opalescence produced in the test solution is not more intense than that in the standard (15 mg/g).

Iron. Dissolve 0.15 g in 5 ml of hydrochloric acid (~70 g/l) TS and dilute to 10 ml with water. Proceed with 4.0 ml of the resulting solution as described under 2.2.4 Limit test for iron; not more than 700 µg/g.

Water-soluble substances. Mix 2.0 g with 100 ml of water and boil for 5 minutes. Filter while still hot, allow to cool, and dilute to 100 ml with water. Evaporate 50 ml of the filtrate to dryness and dry at 105 °C to constant weight; the residue weighs not more than 20 mg.

Substances insoluble in acetic acid. Any residue remaining on the filter used in the preparation of the solution to be examined in the limit test for calcium, when washed with water, dried and ignited at 600 °C, weighs not more than 5 mg.

Loss on ignition. Heat 0.5 g gradually to 900 °C and ignite to constant mass; it loses not less than 0.300 g/g and not more than 0.325 g/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 0.33 g/g.

Assay. Dissolve about 0.05 g, accurately weighed, in 2 ml of hydrochloric acid (~70 g/l) TS and proceed with the titration as described under 2.5 Complexometric titrations for magnesium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.916 mg of $Mg(OH)_2$.

MAGNESII OXIDUM

MAGNESIUM OXIDE

Light magnesium oxide
Heavy magnesium oxide

Molecular formula. MgO

Relative molecular mass. 40.30

Chemical name. Magnesium oxide; CAS Reg. No. 1309-48-4.

Description. A white powder; odourless. Light Magnesium oxide is very bulky, whereas heavy Magnesium oxide is a dense powder.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in dilute acids.

Category. Antacid.

Storage. Magnesium oxide should be kept in a tightly closed container.

Labelling. The designation on the container should state whether it is light Magnesium oxide or heavy Magnesium oxide.

Requirements

Definition. Magnesium oxide contains not less than 98.0% and not more than 100.5% of MgO, calculated with reference to the ignited substance.

Identity tests

A. Dissolve 10 mg in 1.0 ml of hydrochloric acid (~70 g/l) TS, add 1.0 ml of ammonium chloride (100 g/l) TS, 0.5 ml of disodium hydrogen phosphate (100 g/l) TS, and 1.0 ml of ammonia (~100 g/l) TS; a white, crystalline precipitate is formed, which is soluble in acetic acid (~300 g/l) TS.

B. Dissolve 10 mg in 1.0 ml of hydrochloric acid (~70 g/l) TS, and add 2.0 ml of sodium hydroxide (~80 g/l) TS; a white, gelatinous precipitate is produced, which is insoluble in an excess of sodium hydroxide (~80 g/l) TS. Add a few drops of iodine TS; the precipitate turns dark brown.

Heavy metals. Dissolve 5.0 g in a mixture of 70 ml of acetic acid (~300 g/l) TS and 30 ml of water, boil for 2 minutes, cool, and dilute to 100 ml with acetic acid (~120 g/l) TS. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate. To 20 ml of the filtrate (retain the filter for the test of substances insoluble in acetic acid and the remaining filtrate for the limit test for calcium) add 15 ml of hydrochloric acid (~250 g/l) TS and shake with 25 ml of methylisobutylketone R for 2 minutes. Allow to stand, separate the layers, and evaporate the aqueous layer to dryness. Dissolve the residue in 1 ml of acetic acid (~300 g/l) TS and dilute to 40 ml with water and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 40 µg/g.

Arsenic. Use a solution of 3.3 g in 20 ml of sulfuric acid (~100 g/l) TS and 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Barium. To 0.10 g add a few drops of hydrochloric acid (~250 g/l) TS and dissolve in 20 ml of water. Add 0.10 ml of sulfuric acid (~100 g/l) TS and shake; no precipitate is produced within 10 minutes.

Calcium. Dilute 1.3 ml of the filtrate obtained in the test for heavy metals to 150 ml with water.

To 0.20 ml of ethanolic calcium standard (100 µg/ml Ca) TS add 0.8 ml of ammonium oxalate (50 g/l) TS. After 1 minute add 1 ml of acetic acid (~120 g/l) TS and 15 ml of the diluted filtrate prepared above.

Prepare similarly a standard solution using a mixture of 10 ml of calcium standard (10 µg/ml Ca) TS and 5 ml of water. After 15 minutes, any opalescence produced in the test solution is not more intense than that in the standard (15 mg/g).

Iron. Dissolve 0.15 g in 5 ml of hydrochloric acid (~70 g/l) TS and dilute to 10 ml with water. Proceed with 4.0 ml of the resulting solution as described under 2.2.4 Limit test for iron; not more than 500 µg/g.

Water-soluble substances. Mix 2.0 g with 100 ml of water and boil for 5 minutes. While still hot filter through a coarse sintered-glass filter, allow to cool, and dilute to 100 ml with water. Evaporate 50 ml of the filtrate to dryness and dry at 105 °C to constant weight; the residue weighs not more than 20 mg.

Substances insoluble in acetic acid. Any residue remaining on the filter used in the preparation of the solution to be examined in the test for heavy metals, when washed with water, dried, and ignited at 600 °C, weighs not more than 5 mg.

Loss on ignition. Ignite 1.0 g at 900 °C to constant weight; it loses not more than 100 mg/g.

Assay. Dissolve about 0.35 g, accurately weighed, in 20 ml of hydrochloric acid (~70 g/l) TS and proceed with the titration as described under 2.5 Complexometric titrations for magnesium. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.015 mg of MgO.

MAGNESII STEARAS
MAGNESIUM STEARATE

$(H_3C—[CH_2]_{16}—CO_2)_2Mg$

$C_{36}H_{70}MgO_4$

Chemical name. Magnesium stearate; magnesium octadecanoate; CAS Reg. No. 557-04-0.

Description. A white, very fine powder of low bulk density; unctuous and readily adheres to the skin; odour, very faint, of stearic acid.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, and ether R; slightly soluble in hot ethanol (~750 g/l) TS.

Category. Tablet and capsule lubricant; glidant; antiadherent.

Storage. Magnesium stearate should be kept in a well-closed container.

Requirements

Definition. Magnesium stearate consists mainly of magnesium stearate $(C_{17}H_{35}CO_2)_2Mg$ with variable proportions of magnesium palmitate $(C_{15}H_{31}CO_2)_2Mg$ and magnesium oleate $(C_{17}H_{33}CO_2)_2Mg$.

Magnesium stearate contains not less than **3.8%** and not more than the equivalent of **5.8%** of Mg, calculated with reference to the dried substance.

Identity tests

A. To 5 g add 50 ml of ether R, 20 ml of nitric acid (~130 g/l) TS, and 20 ml of water. Heat under a reflux condenser until completely dissolved, and allow to cool. Separate the aqueous layer, shake the ether layer with two quantities, each of 4 ml, of water, combine the aqueous solutions, wash with 15 ml of ether R, and dilute to 50 ml with water. (Retain this solution for identity test B and for "Chlorides".) Evaporate the ether layer to dryness and dry the residue at 105 °C. The congealing point of the residue is not lower than 53 °C. (Keep the residue for "Acid value of fatty acids".)

B. To 1 ml of the above aqueous solution, add 1 ml of ammonia (~100 g/l) TS; a white precipitate is formed which dissolves on the addition of 1 ml of ammonium chloride (100 g/l) TS. Add 1 ml of disodium hydrogen phosphate (40 g/l) TS; a white, crystalline precipitate is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Proceed with 2 ml of the aqueous solution from identity test A as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.25 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 60 mg/g.

Acidity or alkalinity. Boil 1 g with 20 ml of carbon-dioxide-free water R for 1 minute under constant shaking, cool, and filter. To 10 ml of the filtrate add 2 drops of bromothymol blue/ethanol TS and titrate with either hydrochloric

acid (0.1 mol/l) VS or sodium hydroxide (0.1 mol/l) VS; not more than 0.05 ml of either titrant is required to obtain the midpoint of the indicator (green).

Acid value of fatty acids. Use 0.2 g of the residue obtained in identity test A and dissolve in 25 ml of the prescribed mixture of solvents; 195–210.

Assay. With caution, heat gently about 0.5 g, previously dried and accurately weighed, and gradually ignite until a white residue is obtained. Cool, add 10 ml of hydrochloric acid (~70 g/l) TS, and warm on a water-bath for 10 minutes. Dilute with 25 ml of hot water, add sodium hydroxide (~80 g/l) TS until the solution becomes slightly turbid, and then add 10 ml of ammonium chloride buffer, pH 10.0, TS. Proceed with the titration as described under 2.5 Complexometric titrations for magnesium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 1.215 mg of Mg.

MAGNESII SULFATIS HEPTAHYDRAS
MAGNESIUM SULFATE HEPTAHYDRATE

$MgSO_4,7H_2O$

Relative molecular mass. 246.5

Chemical name. Magnesium sulfate (1:1) heptahydrate; CAS Reg. No. 10034-99-8.

Other name. Epsom salt.

Description. Brilliant, colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Cathartic drug.

Storage. Magnesium sulfate heptahydrate should be kept in a well-closed container.

Additional information. Magnesium sulfate heptahydrate effloresces in warm, dry air.

Requirements

Magnesium sulfate heptahydrate contains not less than **99.0%** and not more than the equivalent of **100.5%** of $MgSO_4$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 2 ml of water and add 1 ml of ammonia (~100 g/l) TS; a white precipitate is produced which redissolves after adding 1 ml of ammonium chloride (100 g/l) TS. Then add 1 ml of disodium hydrogen phosphate (40 g/l) TS; a white, fine crystalline precipitate is formed.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Arsenic. Use a solution of 5 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Chlorides. Dissolve 0.85 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 300 µg/g.

Iron. Use 2.0 g; the solution complies with the 2.2.4 Limit test for iron; not more than 20 µg/g.

Clarity and colour of solution. A solution of 1 g in 10 ml of water is clear and colourless.

Loss on drying. Dry 0.5 g at 110–120 °C for 1 hour and then at 400 °C to constant mass; it loses not less than 0.48 g/g and not more than 0.52 g/g.

Acidity or alkalinity. Dissolve 1.0 g in 10 ml of water and add 0.05 ml of phenol red/ethanol TS; not more than 0.2 ml of hydrochloric acid (0.01 mol/l) VS or sodium hydroxide (0.01 mol/l) VS is required to obtain the midpoint of the indicator (pink).

Assay. Dissolve about 0.25 g, accurately weighed, in 100 ml of water, and proceed with the titration as described under 2.5 Complexometric titrations for magnesium.

Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 6.018 mg of $MgSO_4$.

Additional requirements for Magnesium sulfate heptahydrate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.09 IU of endotoxin RS per mg.

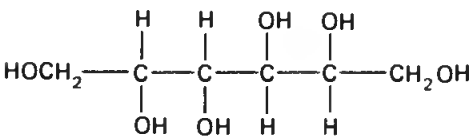
MANNITOLUM

MANNITOL

Molecular formula. $C_6H_{14}O_6$

Relative molecular mass. 182.2

Graphic formula.



Chemical name. D-Mannitol; CAS Reg. No. 69-65-8.

Description. A white, crystalline powder; odourless.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS: practically insoluble in ether R.

Category. Diuretic.

Storage. Mannitol should be kept in a well-closed container.

Additional information. Mannitol has a sweet taste.

Requirements

Definition. Mannitol contains not less than 98.0% and not more than 102.0% of $C_6H_{14}O_6$, calculated with reference to the dried substance.

Identity tests

A. Transfer about 1.0 g, accurately weighed, to a 100-ml volumetric flask, and add 80 ml of ammonium molybdate (45 g/l) TS, previously filtered if neces-

sary. Add sulfuric acid (~50 g/l) TS to volume and mix. Measure the optical rotation and calculate the specific rotation as described under 1.4 Determination of optical rotation and specific rotation; $[\alpha]_D^{20\,°C} = +137$ to $+145\,°$.

B. To 0.5 g add 2.5 ml of acetyl chloride R, then add cautiously 0.5 ml of pyridine R. Warm the mixture until it becomes turbid, cool in ice, and collect the precipitate on a sintered-glass filter. Recrystallize the precipitate several times from ether R and dry at 60 °C for 1 hour; melting temperature, about 123 °C (mannitol hexaacetate).

Melting range. 165–169 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to method A; not more than 10 µg/g.

Arsenic. Use a solution of 5.0 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Chlorides. Dissolve 2.5 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.1 mg/g.

Sulfates. Dissolve 5.0 g in 40 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.1 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Dissolve 5.0 g in 50 ml of carbon-dioxide-free water R and titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS, phenolphthalein/ethanol TS being used as indicator; not more than 0.3 ml is required to obtain the midpoint of the indicator (pink).

Sorbitol. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 85 volumes of 2-propanol R and 15 volumes of a 2 g/l solution of boric acid R as the mobile phase. Prepare a solution of 1.0 g of finely powdered test substance in 10 ml of ethanol (~750 g/l) TS, shake for 30 minutes and filter (solution A). Apply separately to the plate 1 µl of test solution A and 2 µl of a 1.0 mg/ml solution of sorbitol R in water (B).

Develop the plate at room temperature, the process taking up to 5 hours. After removing the plate from the chromatographic chamber, allow it to dry at 110 °C for 5 minutes, cool, and spray with a 1 g/l solution of potassium permanganate R in sulfuric acid (0.5 mol/l) VS. Heat the plate at 110 °C until brown spots appear and examine the chromatogram in daylight. The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

Assay. Dissolve about 0.4 g, accurately weighed, in sufficient water to produce 100 ml. Transfer 10 ml to a stoppered flask, add 20.0 ml of a 21.4 g/l solution of sodium metaperiodate R and 2 ml of sulfuric acid (~100 g/l) TS and heat on a water-bath for 15 minutes. Cool, add 3 g of sodium hydrogen carbonate R, 25 ml of sodium arsenite (0.05 mol/l) VS, and 5 ml of a 200 g/l solution of potassium iodide R; allow to stand for 15 minutes, and titrate with iodine (0.05 mol/l) VS until the first trace of yellow colour appears. Repeat the procedure without the test substance and determine the difference in volume of iodine (0.05 mol/l) VS required for the titration. Each ml of iodine (0.05 mol/l) VS is equivalent to 1.822 mg of $C_6H_{14}O_6$.

Additional requirements for Mannitol for parenteral use

Complies with the monograph for "Parenteral preparations".

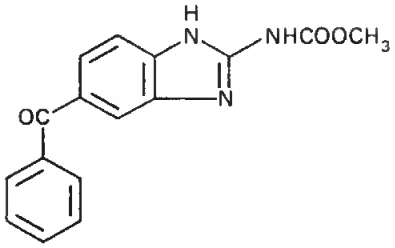
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 4 IU of endotoxin RS per g for dosage forms with a concentration of less than 100 g/l of mannitol, and a limit of not more than 2.5 IU of endotoxin RS per g for dosage forms with a concentration of 100 g/l or more of mannitol.

MEBENDAZOLUM
MEBENDAZOLE

Molecular formula. $C_{16}H_{13}N_3O_3$

Relative molecular mass. 295.3

Graphic formula.



Chemical name. Methyl 5-benzoyl-2-benzimidazolecarbamate; methyl (5-benzoyl-1*H*-benzimidazol-2-yl)carbamate; CAS Reg. No. 31431-39-7.

Description. A white to slightly yellow powder.

Solubility. Practically insoluble in water, dilute mineral acids, ethanol (~750 g/l) TS and ether R; freely soluble in formic acid (~1080 g/l) TS.

Category. Anthelmintic drug.

Storage. Mebendazole should be kept in a well-closed container, protected from light.

Requirements

Definition. Mebendazole contains not less than 98.0% and not more than 102.0% of $C_{16}H_{13}N_3O_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from mebendazole RS or with the *reference spectrum* of mebendazole.

B. Shake 20 mg with 2.0 ml of sodium hydroxide (~80 g/l) TS, and heat the yellowish coloured suspension until it dissolves; the solution is yellow. Add a few drops of copper(II) sulfate (160 g/l) TS; a greenish precipitate is produced. Add a few drops of ammonia (~100 g/l) TS; the colour turns to greenish blue.

C. Dissolve 20 mg in 2 ml of sulfuric acid (~1760 g/l) TS; a yellow solution is produced. Carefully dilute with 3 ml of water; the yellow colour disappears.

Then add 1.0 ml of silver nitrate (40 g/l) TS; a white precipitate is formed, which does not dissolve in an excess of ammonia (~100 g/l) TS.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

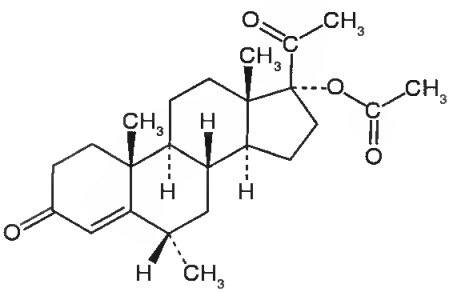
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 (a precoated plate from a commercial source is suitable) as the coating substance and a mixture of 90 volumes of chloroform R, 5 volumes of methanol R, and 5 volumes of formic acid (~1080 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions prepared as follows: (A) Dissolve 50 mg of the test substance in 1.0 ml of formic acid (~1080 g/l) TS in a 10-ml volumetric flask, dilute to volume with chloroform R, and mix; (B) Dilute 1.0 ml of solution A to 200 ml with a mixture of 9 volumes of chloroform R and 1 volume of formic acid (~1080 g/l) TS. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is no larger or more intense than the main spot obtained with solution B.

Assay. Dissolve about 0.22 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 29.53 mg of $C_{16}H_{13}N_3O_3$.

MEDROXYPROGESTERONI ACETAS
MEDROXYPROGESTERONE ACETATE



$C_{24}H_{34}O_4$

Relative molecular mass. 386.5

Chemical name. 17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate; 17-(acetyloxy)-6α-methylpregn-4-ene-3,20-dione; CAS Reg. No. 71-58-9.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in acetone R and dioxan R; slightly soluble in ethanol (~750 g/l) TS, methanol R, and ether R.

Category. Progestogen.

Storage. Medroxyprogesterone acetate should be kept in a well-closed container, protected from light.

Requirements

Medroxyprogesterone acetate contains not less than **97.0%** and not more than the equivalent of **103.0%** of $C_{24}H_{34}O_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from medroxyprogesterone acetate RS or with the *reference spectrum* of medroxyprogesterone acetate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, preparing two plates and using kieselguhr R1 as the coating substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use a mixture of equal volumes of cyclohexane R and light petroleum R1 as the mobile phase. (Keep one plate for the "Related substances".) Apply separately to one plate 2 µl of each of three solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of Medroxyprogesterone acetate per ml, (B) 2.5 mg of medroxyprogesterone acetate RS per ml, and (C) a mixture of equal volumes of solutions A and B. Develop the plate for a distance of 15 cm. After removing the

plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, and spray the hot plate with sulfuric acid/ethanol TS. Heat at 120 °C for a further 10 minutes, allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. A single spot is obtained with solution C.

C. Melting temperature, about 204 °C.

D. Use 20 mg; it yields the reaction described under 2.1 General identification tests as characteristic of acetylated substances.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +45$ to +51 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 3 hours; it loses not more than 10 mg/g.

Related substances. To the plate kept from "Identity test B" and using the same mobile phase, apply separately 5 µl of each of three solutions in chloroform R containing (A) 5 mg of Medroxyprogesterone acetate per ml, (B) 0.15 mg of Medroxyprogesterone acetate per ml, and (C) 0.05 mg of Medroxyprogesterone acetate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 30 minutes, and spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120 °C for 10 minutes, expose the plate to iodine vapours for 10 minutes, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Not more than one such spot is more intense than that obtained with solution C.

Assay. Dissolve about 0.1 g, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 1.0 ml of this solution to 100 ml with the same solvent.

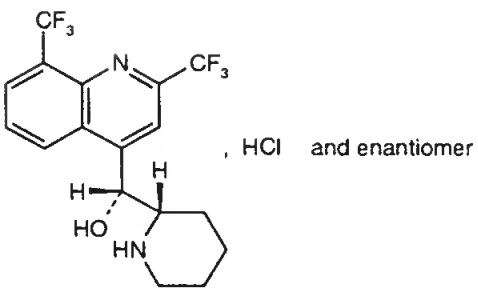
Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 241 nm and calculate the content of $C_{24}H_{34}O_4$ using the absorptivity value of 42.6 ($A_{1\,cm}^{1\%} = 426$).

Additional requirement for Medroxyprogesterone acetate for parenteral use

Complies with the monograph for "Parenteral preparations".

MEFLOQUINI HYDROCHLORIDUM
MEFLOQUINE HYDROCHLORIDE

$C_{17}H_{16}F_6N_2O,HCl$



Relative molecular mass. 414.8

Chemical name. DL-*erythro*-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride; (*R**,*S**)-(±)-α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride; CAS Reg. No. 51773-92-3.

Description. A white to slightly yellow, crystalline powder.

Solubility. Very slightly soluble in water; freely soluble in methanol R; soluble in ethanol (~750 g/l) TS; sparingly soluble in dichloromethane R.

Category. Antimalarial drug.

Storage. Mefloquine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Mefloquine hydrochloride melts at about 260 °C, with decomposition.

Requirements

Mefloquine hydrochloride contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{17}H_{16}F_6N_2O,HCl$, calculated with reference to the anhydrous and solvent-free substance.

Identity tests

- Either tests A and E or tests B, C, D, and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from mefloquine hydrochloride RS or with the *reference spectrum* of mefloquine hydrochloride.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Transfer about 10 mg to a porcelain crucible, add 45 mg of magnesium oxide R, and ignite until an almost white residue is obtained. Allow to cool, add 2.0 ml of water, 0.05 ml of phenolphthalein/ethanol TS, and about 1 ml of hydrochloric acid (~70 g/l) TS. Filter, to the filtrate add a freshly prepared mixture of 0.10 ml of sodium alizarinsulfonate (1 g/l) TS and 0.10 ml of zirconyl nitrate TS, mix, and allow to stand for 5 minutes. Prepare similarly a reagent blank; a yellow colour is produced, whereas the reagent blank is red.

D. To 20 mg add about 0.2 ml of sulfuric acid (~1760 g/l) TS and view the mixture under ultraviolet light (365 nm); a blue fluorescence is observed.

E. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Solution in methanol. A solution of 0.50 g in 10 ml of methanol R is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 1.0 g of Mefloquine hydrochloride; the water content is not more than 30 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 8 volumes of dichloromethane R, 1 volume of glacial acetic acid R1, and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions in methanol R containing (A) 8 mg of Mefloquine

hydrochloride per ml, (B) 1.6mg of Mefloquine hydrochloride per ml, (C) 1.6mg of mefloquine hydrochloride RS per ml, and (D) 0.04mg of mefloquine hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air for 15 minutes, and spray with a freshly prepared mixture of 1 volume of sulfuric acid (~1760g/l) TS and 40 volumes of potassium iodoplatinate TS. Then spray again with hydrogen peroxide (~330g/l) TS and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D (0.5%).

Ethanol, methanol, and acetone. Carry out the test as described under 1.14.5 Gas chromatography, using a stainless steel column (2m × 2.2mm) packed with graphitized carbon (135–175µm) (this may be obtained from a commercial source) which is impregnated with a 0.05g/ml solution of macrogol 20M R. Maintain the column at 70°C, the injection port at 200°C, and the detector at 250°C. Use helium R as the carrier gas at a flow rate of 35ml per minute, and a flame ionization detector.

Use the following two solutions: (1) dissolve 1.0g of Mefloquine hydrochloride in 10ml of dimethylformamide R, and (2) prepare a mixture of 1.0g of methanol R, 1.0g of dehydrated ethanol R, and 1.0g of acetone R diluted to 100ml with dimethylformamide R; further dilute 1.0ml of this solution to 100ml with dimethylformamide R.

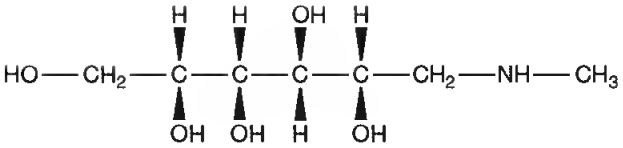
Inject 1µl each of solutions 1 and 2.

Measure the areas of the peak responses obtained in the chromatograms from solutions 1 and 2, and calculate the total content of ethanol, methanol, and acetone; the total content does not exceed 5mg/g.

Assay. Dissolve about 0.31g, accurately weighed, in 70ml of glacial acetic acid R1, add 5ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1mol/l) VS is equivalent to 41.48mg of $C_{17}H_{16}F_6N_2O,HCl$.

MEGLUMINUM
MEGLUMINE



$C_7H_{17}NO_5$

Relative molecular mass. 195.2

Chemical name. 1-Deoxy-1-(methylamino)-D-glucitol; CAS Reg. No. 6284-40-8.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Used in the preparation of meglumine amidotrizoate and meglumine iotroxate as radiocontrast media.

Storage. Meglumine should be kept in a well-closed container.

Requirements

Meglumine contains not less than **99.0%** and not more than the equivalent of **100.5%** of $C_7H_{17}NO_5$, calculated with reference to the dried substance.

Identity tests

A. To 5 ml of water add 0.5 ml of paraldehyde R and 0.5 ml of sulfuric acid (~190 g/l) TS. Shake and warm carefully until a cloudy solution appears, then allow to cool for 15 minutes. Freshly prepare a solution containing 0.1 g of sodium nitroprusside R per ml and to 0.2 ml add 1 ml of the above solution, then add 50 mg of Meglumine and 2 ml of a solution of 50 mg of sodium tetraborate R per ml; a blue colour develops slowly which becomes more intense with time.

B. Dissolve 0.2 g in 2 ml of water, add 0.05 ml of methyl red/ethanol TS, and neutralize with sulfuric acid (0.25 mol/l) VS. Add 1 ml of sodium hydroxide (0.1 mol/l) VS and 1 g of boric acid R; the solution becomes acidic.

Melting range. 128–131 °C.

Specific optical rotation. Use a 0.10 g/ml solution; $[\alpha]_D^{20\,°C} = -15.7$ to -17.3 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Reducing sugars. Dissolve 0.25 g in 5 ml of water, add 5 ml of potassio-cupric tartrate TS and boil for 2 minutes; no red-brown precipitate is produced.

Clarity and colour of solution. A solution of 1 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 10 mg/g.

Assay. Dissolve about 0.5 g, accurately weighed, in 40 ml of water and titrate with hydrochloric acid (0.1 mol/l) VS, using methyl red/ethanol TS as indicator.

Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 19.52 mg of $C_7H_{17}NO_5$.

Additional requirement for Meglumine for parenteral use

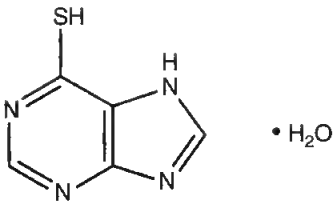
Complies with the monograph for "Parenteral preparations".

Pyrogens. Carry out the test as described under 3.5 Test for pyrogens, injecting, per kg of the rabbit's mass, a solution in sterile water R containing 0.6 g of Meglumine in not more than 5 ml.

Additional requirement for Meglumine for sterile use

Complies with 3.2.1 Test for sterility of non-injectable preparations.

MERCAPTOPURINUM
MERCAPTOPURINE



• H_2O

$C_5H_4N_4S,H_2O$

Relative molecular mass. 170.2

Chemical name. Purine-6-thiol monohydrate; 1,7-dihydro-6*H*-purine-6-thione monohydrate; CAS Reg. No. 6112-76-1.

Description. A yellow, crystalline powder.

Solubility. Practically insoluble in water and ether R; slightly soluble in ethanol (~750 g/l) TS; dissolves in solutions of alkali hydroxides.

Category. Cytotoxic drug.

Storage. Mercaptopurine should be kept in a well-closed container, protected from light.

Additional information. *CAUTION:* Mercaptopurine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.
It melts at a temperature exceeding 308 °C with decomposition.

Requirements

Mercaptopurine contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_5H_4N_4S$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 20 mg in 5 ml of dimethyl sulfoxide R and dilute to 100 ml with hydrochloric acid (0.1 mol/l) VS. Dilute 5 ml of this solution to 200 ml with hydrochloric acid (0.1 mol/l) VS. The absorption spectrum of the diluted solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 325 nm.

B. Dissolve 20 mg in 20 ml of warm ethanol (~750 g/l) TS and add 1 ml of a saturated solution of mercuric acetate R in ethanol (~750 g/l) TS; a white precipitate is produced.

C. Dissolve 20 mg in 20 ml of warm ethanol (~750 g/l) TS and add 1 ml of a solution containing 10 mg of lead acetate R per ml of ethanol (~750 g/l) TS; a yellow precipitate is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.15 g; the water content is not less than 100 mg/g and not more than 120 mg/g.

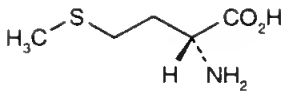
Hypoxanthine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 90 volumes of acetone R, 7 volumes of water, and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of two solutions containing (A) 50 mg of Mercaptopurine dissolved in 1 ml of dimethyl sulfoxide R and diluted to 10 ml with methanol R, and (B) 10 mg of hypoxanthine R dissolved in 10 ml of dimethyl sulfoxide R and diluted to 100 ml with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of dimethylformamide R, add 5 drops of thymol blue/dimethylformamide TS, and titrate with sodium methoxide (0.1 mol/l) VS to a blue endpoint, as described under 2.6 Non-aqueous titration, Method B.

Each ml of sodium methoxide (0.1 mol/l) VS is equivalent to 15.22 mg of $C_5H_4N_4S$.

DL-METHIONINUM
DL-METHIONINE



and enantiomer

$C_5H_{11}NO_2S$

Relative molecular mass. 149.2

Chemical name. (*RS*)-2-Amino-4-(methylthio)butyric acid; CAS Reg. No. 59-51-8.

Description. An almost white, crystalline powder or small flakes.

Solubility. Sparingly soluble in water; very slightly soluble in ethanol (~750 g/l) TS. It dissolves in dilute acids and in dilute solutions of the alkali hydroxides.

Category. Antidote.

Storage. DL-Methionine should be protected from light.

Additional information. Melting temperature, about 270 °C.

Requirements

DL-Methionine contains not less than **99.0%** and not more than **101.0%** of $C_5H_{11}NO_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from DL-methionine RS or with the *reference spectrum* of DL-methionine.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Dissolve together about 0.1 g of DL-Methionine with 0.1 g of glycine R in 4.5 ml of sodium hydroxide (~80 g/l) TS, add 1 ml of 25 mg/ml solution of

sodium nitroprusside R, heat at 40 °C for 10 minutes, and allow to cool. Add 2 ml of a mixture of 1 volume of phosphoric acid (~1440 g/l) TS and 9 volumes of hydrochloric acid (~420 g/l) TS; a deep-red colour is produced.

D. Use a 0.050 g/ml solution in hydrochloric acid (1 mol/l) VS and measure the angle of optical rotation as described under 1.4 Determination of optical rotation and specific rotation; $[\alpha]_D^{20\,°C} = -0.05°$ to $+0.05°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 1.2 g in a mixture of 5 ml of nitric acid (~130 g/l) TS and 35 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfates. Dissolve 1.0 g in 20 ml of water for injections R by heating to 60 °C, cool to 10 °C, and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of carbondioxide-free water R is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 5.4–6.1.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 6 volumes of 2-butanol R, 2 volumes of glacial acetic acid R, and 2 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions containing (A) 20 mg of DL-Methionine per ml, (B) 0.40 mg of DLMethionine per ml, (C) 0.40 mg of DL-methionine RS per ml, and (D) 0.040 mg of DL-methionine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with triketohydrindene/butanol/acetic acid TS and heat at 105 °C for 15 minutes. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D (0.2%).

Assay. Dissolve about 0.14 g, accurately weighed, in 3 ml of anhydrous formic acid R and add 30 ml of glacial acetic acid R1. Without delay titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

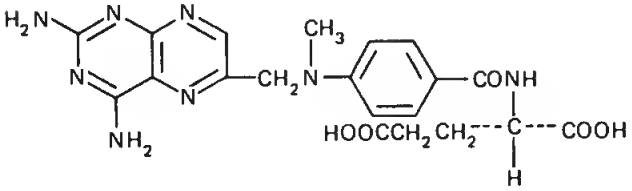
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

METHOTREXATUM
METHOTREXATE

Molecular formula. $C_{20}H_{22}N_8O_5$

Relative molecular mass. 454.4

Graphic formula.



Chemical name. (+)-*N*-[*p*-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino] benzoyl]-L-glutamic acid; *N*-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid; CAS Reg. No. 59-05-2.

Description. A yellow to orange, crystalline powder.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, dichloroethane R, and ether R; very soluble in diluted solutions of alkali hydroxides and carbonates.

Category. Cytotoxic drug.

Storage. Methotrexate should be kept in a tightly closed container, protected from light.

Additional information. Methotrexate is gradually affected by light. CAUTION: Methotrexate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Methotrexate contains not less than 96.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from methotrexate RS or with the *reference spectrum* of methotrexate.

B. The absorption spectrum of a 10.0 µg/ml solution in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 380 nm, exhibits 3 maxima at about 258 nm, 303 nm, and 371 nm. The ratio of the absorbance at 303 nm to that at 371 nm is between 2.8 and 3.3.

Specific optical rotation. Dissolve 0.25 g in 12 ml of sodium carbonate (10 g/l) TS, dilute with water to 25 ml, and calculate the result with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +19 to +24 °.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 120 mg/g.

Assay. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a column 10 cm long and 6 mm in internal diameter packed with silica gel, 5 µm in diameter, the surface of which has been modified with chemically bonded octadecylsilyl groups.

As the mobile phase, use a mixture of 8 volumes of acetonitrile R with 92 volumes of phosphate/citrate buffer pH 6.0, TS.

Prepare the following solutions in the above-mentioned mobile phase containing (A) 0.10 mg of the test substance per ml, (B) 0.10 mg of methotrexate RS per ml, and (C) 0.10 mg of methotrexate RS and 0.10 mg of folic acid RS per ml for the system suitability test.

Operate at room temperature with a flow rate of about 1.4 ml per minute. As a detector use an ultraviolet spectrophotometer at a wavelength of about 303 nm, fitted with a low-volume flow cell (10 µl is suitable), and a suitable recorder.

Make 6 replicate injections of solution C, each of 20 µl. The resolution factor between methotrexate and folic acid should be not less than 5.0, with a relative standard deviation for the methotrexate peak of not more than 2.5% (adjust the flow rate and the ratio of the mobile phase if it does not conform).

Inject 20 µl of each of solutions A and B. Measure the peak responses and calculate the content in % of $C_{20}H_{22}N_8O_5$ using the following formula:

$100(A_1M_2T)/(A_2M_1)$, in which A_1 and A_2 are the peak responses of the test substance and the reference substance, respectively, and M_1 and M_2 are the concentrations of the test solution and the reference solution, respectively, and T corresponds to the degree of purity of methotrexate RS.

Additional requirement for Methotrexate for parenteral use

Complies with the monograph for "Parenteral preparations".

METHYLCELLULOSUM
METHYLCELLULOSE

Chemical name. Cellulose methyl ether; CAS Reg. No. 9004-67-5.

Description. A white, yellowish white, or greyish white powder or loose, cotton-like, fibrous material; odourless.

Solubility. Practically insoluble in hot water, ethanol (~750 g/l) TS, ether R and acetone R; soluble in glacial acetic acid R and in a mixture of equal volumes of ethanol (~750 g/l) TS and chloroform R.

Category. Coating agent; viscosity-increasing agent; tablet binder.

Storage. Methylcellulose should be kept in a well-closed container.

Labelling. The designation on the container of Methylcellulose should state its viscosity.

Additional information. Methylcellulose is hygroscopic after drying. In cold water it swells and produces a clear to opalescent, viscous, colloidal suspension which is neutral to litmus R.

Requirements

Definition. Methylcellulose is a methyl ether of cellulose.

Methylcellulose contains not less than **26.0%** and not more than the equivalent of **32.0%** of methoxy ($-OCH_3$) groups.

Identity tests

A. While stirring, add 1 g of dried Methylcellulose to 50 ml of carbon-dioxide-free water R heated to 90 °C. Allow to cool, dilute to 100 ml with the same

solvent, and stir until completely dissolved. Heat 10 ml in a water-bath while stirring (keep the remaining solution for test B); above 50 °C a cloudy solution or a flocculent precipitate is formed, and on cooling the solution becomes clear.

B. Place 1 ml of the above solution on a glass plate and allow to evaporate; a thin film is formed.

C. Dissolve without heating 0.2 g in 15 ml of sulfuric acid (~1125 g/l) TS. Pour the solution with stirring into 100 ml of ice-water, and dilute to 250 ml with ice-water. While cooling in ice-water, mix thoroughly in a test-tube 1 ml of the prepared solution with 8 ml of sulfuric acid (~1760 g/l) TS, added drop by drop. Heat in a water-bath for exactly 3 minutes and immediately cool in ice-water. While cold, carefully add 0.6 ml of triketohydrindene/sodium metabisulfite TS and mix well. Allow to stand at 25 °C; a pink colour is immediately produced, which does not change to violet within 100 minutes.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 10 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 100 mg/g.

Assay. Carry out the assay as described under 2.9 Determination of methoxyl, using about 0.05 g, previously dried and accurately weighed.

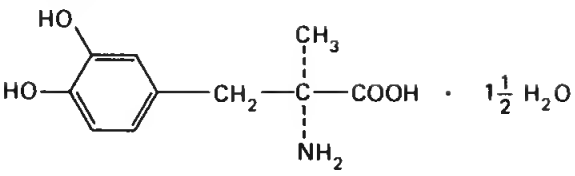
Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 0.5172 mg of ($-OCH_3$).

METHYLDOPUM
METHYLDOPA

Molecular formula. $C_{10}H_{13}NO_4,1\frac{1}{2}H_2O$

Relative molecular mass. 238.2

Graphic formula.



Chemical name. L-3-(3,4-Dihydroxyphenyl)-2-methylalanine sesquihydrate; 3-hydroxy-α-methyl-L-tyrosine sesquihydrate; CAS Reg. No. 41372-08-1.

Description. White to yellowish white, fine powder or lumps; odourless.

Solubility. Slightly soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antihypertensive.

Storage. Methyldopa should be kept in a well-closed container, protected from light.

Requirements

Definition. Methyldopa contains not less than 98.0% and not more than 101.0% of $C_{10}H_{13}NO_4$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from methyldopa RS or with the *reference spectrum* of methyldopa.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R2 as the coating substance and a mixture of 50 volumes of 1-butanol R, 25 volumes of glacial acetic acid R and 25 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in hydrochloric acid (1 mol/l) VS containing (A) 10 mg of the test substance per ml and (B) 10 mg of methyldopa RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, spray with a freshly prepared solution composed of 2 volumes of ferric chloride (25 g/l) TS and 1 volume of potassium ferricyanide (50 g/l) TS, and examine the chromatogram in daylight. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To 5 mg add 1 ml of water, 1 ml of pyridine R, and 5 mg of 4-nitrobenzoyl chloride R and heat to boiling. While shaking, add 0.1 ml of sodium carbonate (200 g/l) TS; an orange or amber colour is produced.

Specific optical rotation. Use a 44 mg/ml solution in aluminium chloride TS and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = -25$ to $-28\,°$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

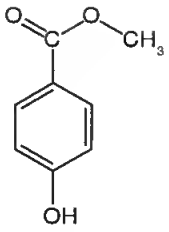
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method. Method A, using about 0.2 g of the substance; the water content is not less than 100 mg/g and not more than 130 mg/g.

Acidity. Dissolve 1.0 g in 100 ml of carbon-dioxide-free water R with the aid of heat and titrate with sodium hydroxide (0.1 mol/l) VS, methyl red/ethanol TS being used as indicator; not more than 0.5 ml is required to obtain the mid-point of the indicator (orange).

3-*O*-Methyl derivative. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R2 as the coating substance and a mixture of 65 volumes of 1-butanol R, 15 volumes of glacial acetic acid R, and 25 volumes of water as the mobile phase. Apply separately to the plate (A) 10 µl of a 10 mg/ml solution of the test substance dissolved in a mixture of 4 volumes of hydrochloric acid (~250 g/l) TS and 96 volumes of methanol R, (B) 10 µl of a 50 µg/ml solution of (–)-3-(4-hydroxy-3-methoxyphenyl)-2-methylalanine RS, and (C) 20 µl of a mixture of equal volumes of solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and spray with a mixture of 5 volumes of a 0.05 g/ml solution of sodium nitrite R and 45 volumes of a 3 mg/ml solution of 4-nitroaniline R dissolved in a mixture of 80 volumes of hydrochloric acid (~420 g/l) TS and 20 volumes of water. Dry in a current of warm air, spray with sodium carbonate (75 g/l) TS, and examine the chromatogram in daylight. The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A. The test is valid only if the chromatogram obtained with solution C shows two distinctly separated spots.

Assay. Dissolve about 0.20 g, accurately weighed, in 20 ml of glacial acetic acid R1, add 20 ml of dioxan R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 21.12 mg of $C_{10}H_{13}NO_4$.

METHYLIS HYDROXYBENZOAS
METHYL HYDROXYBENZOATE



$C_8H_8O_3$

Relative molecular mass. 152.2

Chemical name. Methyl *p*-hydroxybenzoate; methyl 4-hydroxybenzoate; CAS Reg. No. 99-76-3.

Other name. Methylparaben.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; soluble in boiling water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Antimicrobial preservative.

Storage. Methyl hydroxybenzoate should be kept in a well-closed container.

Additional information. Methyl hydroxybenzoate is normally used in combination with other hydroxybenzoates.

Requirements

Methyl hydroxybenzoate contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_8H_8O_3$, calculated with reference to the dried substance.

Identity tests

A. Complies with the test under "Melting range".

B. To 0.5 g add 5 ml of sodium hydroxide (~80 g/l) TS, and heat in a water-bath for 5 minutes. After cooling, add 6 ml of sulfuric acid (~190 g/l) TS, collect the precipitate on a filter, wash thoroughly with a small amount of water, and dry over silica gel, desiccant, R. Melting temperature, about 214 °C.

Melting range. 125–128 °C.

Sulfated ash. 1.0 mg/g.

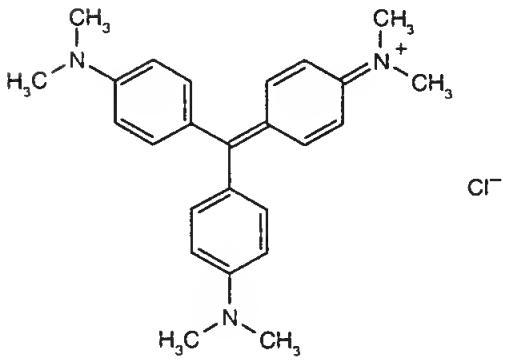
Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 2 hours; it loses not more than 5.0 mg/g.

Acidity. Dissolve 0.2 g in 5 ml of ethanol (~750 g/l) TS, add 5 ml of carbon-dioxide-free water R, and titrate with sodium hydroxide (0.1 mol/l) VS, using 0.1 ml of bromocresol green/ethanol TS as indicator; not more than 0.1 ml is required to obtain the midpoint of the indicator (green).

Assay. Place about 80 mg, accurately weighed, in a ground-glass-stoppered flask, add 25 ml of sodium hydroxide (~80 g/l) TS, and boil gently under a reflux condenser for 30 minutes. Allow to cool, add 25 ml of potassium bromate (0.0333 mol/l) VS, 5 ml of potassium bromide (125 g/l) TS, and 40 ml of glacial acetic acid R. Cool in ice-water and add 10 ml of hydrochloric acid (~420 g/l) TS. Stopper the flask immediately and allow to stand for 15 minutes. Add 30 ml of potassium iodide (80 g/l) TS, close the flask, and mix. Titrate with sodium thiosulfate (0.1 mol/l) VS, using 2 ml of starch TS as indicator, added towards the end of the titration. Repeat the procedure without the Methyl hydroxybenzoate being examined and make any necessary corrections.

One volume of sodium thiosulfate (0.1 mol/l) VS corresponds to two volumes of potassium bromate (0.0333 mol/l) VS. Each ml of potassium bromate (0.0333 mol/l) VS is equivalent to 5.073 mg of $C_8H_8O_3$.

METHYLROSANILINII CHLORIDUM
METHYLROSANILINIUM CHLORIDE



$C_{25}H_{30}ClN_3$

Relative molecular mass. 408.0

Chemical name. C.I. Basic violet 3; *N*-[4-[bis[4-(dimethylamino)phenyl] methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride; CAS Reg. No. 548-62-9.

Other names. Crystal violet, gentian violet.

Description. A dark green powder or greenish, glistening pieces having a metallic lustre; odourless or almost odourless.

Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/l) TS and glycerol R; practically insoluble in ether R.

Category. Anti-infective drug.

Storage. Methylrosanilinium chloride should be kept in a tightly closed container, protected from light.

Requirements

Methylrosanilinium chloride contains not less than **96.0%** and not more than the equivalent of **101.0%** of $C_{25}H_{30}ClN_3$, calculated with reference to the anhydrous substance.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds to the spot with the lowest R_f-value of the three distinct spots obtained with solution B. A spot other than the principal spot may be present on the chromatogram obtained with solution A; this other spot corresponds to the spot with intermediate R_f-value obtained with solution B.

B. Dissolve about 20 mg in 10 ml of water and add 5 drops of hydrochloric acid (~420 g/l) TS. To 5 ml of this solution add tannic acid (50 g/l) TS, drop by drop; a blue precipitate is produced (keep the remaining solution for test C).

C. To the remaining solution from test B add 0.5 g of zinc R powder, and warm the mixture; the solution discolours rapidly. Place on a filter-paper 1 drop of this solution adjacent to 1 drop of ammonia (~100 g/l) TS; a blue colour is produced at the zone of contact.

Ethanol-insoluble matter. Add 1.0 g to 50 ml of ethanol (~750 g/l) TS and boil under reflux for 15 minutes. Filter through a tared filtering crucible, wash the residue on the filter with hot ethanol (~750 g/l) TS until no violet colour appears

in the washings, and dry the crucible at 105 °C for 1 hour; the residue weighs not more than 10 mg (1.0%).

Sulfated ash. Not more than 15 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g of Methylrosanilinium chloride; the water content is not more than 0.075 g/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 100 volumes of 1-butanol R, 5 volumes of ammonium chloride (20 g/l) TS, and 0.5 volume of formic acid (~1080 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 1 mg of Methylrosanilinium chloride per ml, and (B) 1 mg of methyl violet 2B R per ml; also apply to the plate 10 µl of each of 4 solutions in methanol R containing (C) 10 mg of Methylrosanilinium chloride per ml, (D) 2.5 mg of Methylrosanilinium chloride per ml, (E) 0.05 mg of 4,4′-bis(dimethylamino) benzophenone R per ml, and (F) 0.05 mg of Methylrosanilinium chloride per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution C corresponding to 4,4′-bis(dimethylamino) benzophenone is not more intense than that obtained with solution E (0.5%). Any spot obtained with solution D, other than the principal spot or any spot due to 4,4′-bis(dimethylamino) benzophenone, is not more intense than that obtained with solution F (2.0%).

Assay. Transfer about 0.4 g, accurately weighed, to a 300-ml conical flask, add 25 ml of water and 10 ml of hydrochloric acid (~420 g/l) TS. Replace the air in the flask with carbon dioxide R and maintain a stream of carbon dioxide R through the flask during the determination. Add 50.0 ml of titanium trichloride (0.1 mol/l) VS, heat to boiling, continuing to boil gently for 10 minutes, swirling the liquid occasionally. Cool, add 5 ml of ammonium thiocyanate (10 g/l) TS, and titrate with ferric ammonium sulfate (0.1 mol/l) VS until a faint red colour is produced. Repeat the procedure without the Methylrosanilinium chloride being examined and make any necessary corrections.

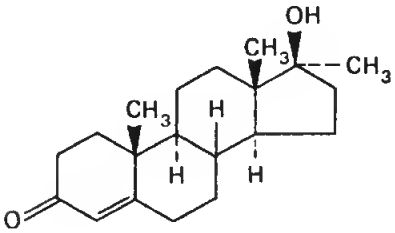
Each ml of titanium trichloride (0.1 mol/l) VS is equivalent to 20.40 mg of $C_{25}H_{30}ClN_3$.

METHYLTESTOSTERONUM
METHYLTESTOSTERONE

Molecular formula. $C_{20}H_{30}O_2$

Relative molecular mass. 302.5

Graphic formula.



Chemical name. 17β-Hydroxy-17-methylandrost-4-en-3-one; CAS Reg. No. 58-18-4.

Description. Colourless or almost colourless crystals or a white or slightly yellowish white, crystalline powder; odourless.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS; sparingly soluble in ether R.

Category. Androgen.

Storage. Methyltestosterone should be kept in a well-closed container, protected from light.

Requirements

Definition. Methyltestosterone contains not less than 97.0% and not more than 102.0% of $C_{20}H_{30}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from methyltestosterone RS or with the *reference spectrum* of methyltestosterone.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of propylene glycol R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 80 volumes of cyclohexane R and 20 volumes of toluene R. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 1.0 mg of the test substance per ml and (B) 1.0 mg of methyltestosterone RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with 4-toluenesulfonic acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Melting temperature, about 165 °C.

Specific optical rotation. Use a 10 mg/ml solution in ethanol (~750 g/l) TS; $[\alpha]_D^{20\,°C} = +78$ to $+85°$.

Solution in ethanol. A solution of 0.50 g in 10 ml of ethanol (~750 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 90 volumes of chloroform R and 10 volumes of acetone R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 10 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the

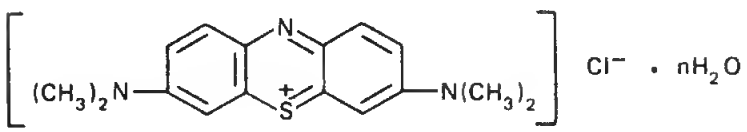
same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 242 nm. Calculate the amount of $C_{20}H_{30}O_2$ in the substance being tested by comparison with methyltestosterone RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.54 ± 0.03.

METHYLTHIONINII CHLORIDUM
METHYLTHIONINIUM CHLORIDE

Molecular formula. $C_{16}H_{18}ClN_3S$ (anhydrous); $C_{16}H_{18}ClN_3S,3H_2O$ (trihydrate).

Relative molecular mass. 319.9 (anhydrous); 373.9 (trihydrate).

Graphic formula.



n = 0 (anhydrous)
n = 3 (trihydrate)

Chemical name. C.I. Basic Blue 9; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride; CAS Reg. No. 61-73-4 (anhydrous).

C.I. Basic Blue 9 trihydrate; 3,7-bis(dimethylamino)phenothiazin-5-ium chloride trihydrate; CAS Reg. No. 7220-79-3 (trihydrate).

Other name. Methylene blue.

Description. Dark green crystals with a metallic lustre or a dark green, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antidote.

Storage. Methylthioninium chloride should be kept in a tightly closed container, protected from light.

Additional information. Methylthioninium chloride is hygroscopic.

Requirements

Definition. Methylthioninium chloride contains not less than 97.0% and not more than 101.0% of $C_{16}H_{18}ClN_3S$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 5 µg/ml solution in hydrochloric acid (~70 g/l) TS, when observed between 230 nm and 800 nm, exhibits 4 maxima at about 258 nm, 288 nm, 680 nm, and 745 nm.

B. Dissolve 1 mg in 10 ml of water; a deep blue colour is produced. Add 2.0 ml of hydrochloric acid (~70 g/l) TS and 0.25 g of zinc R powder; the colour of the solution is discharged; filter and expose the filtrate to the air; the blue colour of the solution reappears.

C. Mix 0.05 g with 0.5 g of anhydrous sodium carbonate R in a porcelain crucible. Carefully heat the mixture to a red glow for 10 minutes. Cool, dissolve the residue in 10 ml of nitric acid (~130 g/l) TS and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Copper or zinc. Ignite 1.0 g in a porcelain crucible using as low a temperature as practicable, until all of the carbon is oxidized. Cool the residue, add 15 ml of nitric acid (~130 g/l) TS and boil for 5 minutes. Separately prepare a reference solution by boiling a quantity of copper(II) sulfate R, equivalent to 200 µg of Cu, with 15 ml of nitric acid (~130 g/l) TS for 5 minutes. Filter separately the cooled test and reference solutions, and wash any residue with 10 ml of water. Combine the filtrate and washings of the test solution and similarly combine the filtrate and washings of the reference solution; add to each an excess of ammonia (~100 g/l) TS and filter the solutions into 50-ml volumetric flasks. Wash the precipitates with small portions of water, adding the washings to the filtrates; dilute the contents of each flask with water to volume, mixing thoroughly. To 25 ml of each of the solutions add 10 ml of hydrogen sulfide TS; no turbidity is produced within 5 minutes (absence of zinc) and any dark colour produced in the test solution is not more intense than that of the reference solution (the copper content is not more than 0.20 mg/g).

Iron. Mix 4 g with 200 ml of water in a long-necked, round-bottomed flask, add 15 ml of nitric acid (~1000 g/l) TS, bring gently to the boil and continue boiling until the volume of liquid is reduced to about 20 ml. Allow to cool, add 10 ml of sulfuric acid (~1760 g/l) TS and mix. Heat to boiling and add small successive quantities of nitric acid (~1000 g/l) TS, cooling before each addition, until a colourless liquid is obtained. Heat until white fumes are evolved; if darkening occurs at this stage continue the treatment with nitric acid (~1000 g/l) TS, finally heating until white fumes are again evolved. Allow the colourless liquid

to cool, add 25 ml of a saturated solution of ammonium oxalate R in water, and boil until the slight froth completely subsides. Cool, dilute to 50 ml with water; 5 ml of the diluted solution complies with the 2.2.4 Limit test for iron; not more than 0.10 mg/g.

Sulfated ash. Not more than 10 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 80 mg/g and not more than 220 mg/g.

Foreign dyes. Carry out the test as described under 1.14.1 Thin-layer chromatography, using as the coating substance a slurry prepared from silica gel R1 and a mixture of equal volumes of potassium dihydrogen phosphate (27.2 g/l) TS and disodium hydrogen phosphate (28.4 g/l) TS. As the mobile phase, use a mixture of 20 volumes of 1-propanol R, 4 volumes of anhydrous formic acid R, and 1 volume of water. Apply to the plate 2 µl of a solution prepared by dissolving 25 mg of the test substance in sufficient methanol R to produce 10 ml. After removing the plate from the chromatographic chamber, allow it to dry in an oven at 105 °C. At an R_f value of about 0.5, 3–4 spots appear, placed very close to each other, the lowest spot being violet in colour and the others red, the intensity of the colour increasing in ascending order of the spots. No other spot is detected.

Assay. Transfer about 0.3 g, accurately weighed, to a 100-ml volumetric flask, dissolve in 30 ml of water by warming on a water-bath, and allow the solution to cool. While shaking, add 50.0 ml of potassium dichromate (0.0167 mol/l) VS, dilute to volume with water, and mix. Repeat the shaking intermittently for 10 minutes, and filter; discard the first 20 ml of the filtrate. Transfer 50.0 ml of the filtrate to a glass-stoppered flask, add 40 ml of sulfuric acid (~190 g/l) TS and 1 g of potassium iodide R, mix, and allow the closed flask to stand in the dark for 5 minutes. Add 100 ml of water and titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, until a blue-green colour is obtained. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of potassium dichromate (0.0167 mol/l) VS is equivalent to 10.66 mg of $C_{16}H_{18}ClN_3S$.

Additional requirements for Methylthioninium chloride for parenteral use

Complies with the monograph for "Parenteral preparations".

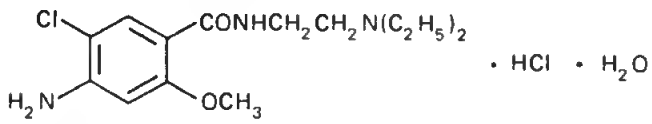
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.5 IU of endotoxin RS per mg.

METOCLOPRAMIDI HYDROCHLORIDUM
METOCLOPRAMIDE HYDROCHLORIDE

Molecular formula. $C_{14}H_{22}ClN_3O_2,HCl,H_2O$

Relative molecular mass. 354.3

Graphic formula.



Chemical name. 4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-*o*-anisamide monohydrochloride monohydrate; 4-amino-5-chloro-*N*-[2-(diethylamino)-ethyl]-2-methoxybenzamide monohydrochloride monohydrate; CAS Reg. No. 54143-57-6 (monohydrate).

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiemetic drug.

Storage. Metoclopramide hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Metoclopramide hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{14}H_{22}ClN_3O_2,HCl$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from metoclopramide hydrochloride RS or with the *reference spectrum* of metoclopramide hydrochloride.

B. The absorption spectrum of a 20 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 230 nm and 350 nm, exhibits

maxima at about 273 nm and 309 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.79 and 0.69, respectively.

C. Dissolve 0.05 g in 5 ml of water and add 5 ml of 4-dimethylaminobenzaldehyde TS5; a yellow-orange colour is produced.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not less than 45 mg/g and not more than 55 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 95 volumes of 1-butanol R and 5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.50 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of acetic anhydride R, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.63 mg of $C_{14}H_{22}ClN_3O_2$,HCl.

Additional requirements for Metoclopramide hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

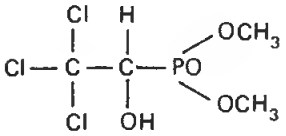
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.5 IU of endotoxin RS per mg of metoclopramide.

METRIFONATUM
METRIFONATE

Molecular formula. $C_4H_8Cl_3O_4P$

Relative molecular mass. 257.4

Graphic formula.



Chemical name. Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate; CAS Reg. No. 52-68-6.

Description. A white or yellowish white, crystalline powder.

Solubility. Sparingly soluble in water; very soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antischistosomal drug.

Storage. Metrifonate should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Metrifonate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. CAUTION: Metrifonate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Metrifonate contains not less than 95.0% and not more than 101.0% of $C_4H_8Cl_3O_4P$, calculated with reference to the anhydrous substance.

Identity tests

A. In a porcelain crucible mix 0.1 g with 0.2 g of a mixture composed of equal parts of potassium nitrate R and anhydrous sodium carbonate R, heat it carefully to a red glow for 3 minutes, cool the residue, and dissolve it in a mixture of 7 ml of water and 3 ml of nitric acid (~1000 g/l) TS. Keep 2 ml of this solution for test B. To 5 ml add 2 ml of ammonium molybdate (95 g/l) TS and heat to boiling; a yellow, crystalline precipitate is produced.

B. To 2 ml of the solution kept in test A add 1 ml of silver nitrate (0.1 mol/l) VS; a white precipitate is produced, which is soluble in 3 ml of ammonia (~100 g/l) TS.

Congealing temperature. Melt 6.2 g in a water-bath at 85 °C, add 2 g of calcium sulfate R, heat again to 80 °C, and proceed as described under 1.2.2 Congealing point; not below 73.5 °C.

Acetone-insoluble matter. Shake 1.0 g with 20 ml of acetone R for 15 minutes, filter, wash the filter with 10 ml of acetone R and dry it at 105 °C for 1 hour; the content of insoluble matter is not more than 5.0 mg/g.

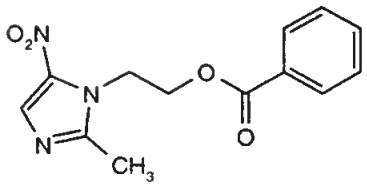
Sulfated ash. Not more than 3.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 7.5 mg/g.

pH value. pH of a 5.0 mg/ml solution, 2.0–3.5.

Assay. Dissolve about 1.0 g, accurately weighed, in 90 ml of methanol R. Add 10 ml of monoethanolamine R and allow the solution to stand at 20 °C ± 0.5 °C for 1 hour. Then cool it in an ice-bath for 10 minutes, add 20 ml of nitric acid (~1000 g/l) TS, and titrate at 20 °C with silver nitrate (0.1 mol/l) VS, determining the endpoint potentiometrically using a platinum electrode and a calomel reference electrode. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 25.74 mg of $C_4H_8Cl_3O_4P$.

METRONIDAZOLI BENZOAS
METRONIDAZOLE BENZOATE



$C_{13}H_{13}N_3O_4$

Relative molecular mass. 275.3

Chemical name. 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate; 2-methyl-5-nitro-1*H*-imidazole-1-ethanol benzoate; CAS Reg. No. 13182-89-3.

Description. A white or slightly yellowish, crystalline powder.

Solubility. Practically insoluble in water; freely soluble in dichloromethane R; soluble in acetone R; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Anti-infective drug.

Storage. Metronidazole benzoate should be kept in a well-closed container, protected from light.

Requirements

Metronidazole benzoate contains not less than **98.5%** and not more than **101.0%** of $C_{13}H_{13}N_3O_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from metronidazole benzoate RS or with the *reference spectrum* of metronidazole benzoate.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. To about 10 mg add 10 mg of zinc R powder, 1 ml of water, and about 0.3 ml of hydrochloric acid (~420 g/l) TS. Heat on a water-bath for 5 minutes and cool. The solution yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a red precipitate.

D. Melting temperature, about 101 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 80 °C for 3 hours; it loses not more than 5.0 mg/g.

pH value. pH of a 20 mg/ml suspension in carbon-dioxide-free water R, 5.0–7.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance. Heat to activate the plate at 110 °C for 1 hour and cool before use. As the mobile phase, use ethyl acetate R. Apply separately to the plate 10 µl of each of 8 solutions in acetone R containing (A) 20 mg of Metronidazole benzoate per ml, (B) 2.0 mg of Metronidazole benzoate per ml, (C) 2.0 mg of metronidazole benzoate RS per ml, (D) 0.10 mg of Metronidazole benzoate per ml, (E) 0.040 mg of Metronidazole benzoate per ml, (F) 0.10 mg of metronidazole RS per ml, (G) 0.10 mg of 2-methyl-5-nitroimidazole R per ml, and for solution (H) dissolve 10 mg of metronidazole RS and 10 mg of 2-methyl-5-nitroimidazole R in sufficient acetone R to produce 50 ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot corresponding to metronidazole or to 2-methyl-5-nitroimidazole obtained with solution A is not more intense than the corresponding spot obtained with solutions F and G (0.5%). Any spot obtained with solution A, other than the principal spot and the spots corresponding to metronidazole and to 2-methyl-5-nitroimidazole, is not more intense than that obtained with solution D (0.5%), and not more than one such spot is more intense than that obtained with solution E (0.2%). The test is not valid unless the chromatogram obtained with solution H shows two clearly separated principal spots.

Assay. Dissolve about 0.25 g, accurately weighed, in 50 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

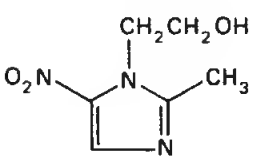
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.53 mg of $C_{13}H_{13}N_3O_4$.

METRONIDAZOLUM
METRONIDAZOLE

Molecular formula. $C_6H_9N_3O_3$

Relative molecular mass. 171.2

Graphic formula.



Chemical name. 2-Methyl-5-nitroimidazole-1-ethanol; 2-methyl-5-nitro-1*H*-imidazole-1-ethanol; CAS Reg. No. 443-48-1.

Description. A white or pale yellow, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; slightly soluble in ethanol (~750 g/l) TS, and ether R.

Category. Antitrichomonal; antiamoebic.

Storage. Metronidazole should be kept in a well-closed container, protected from light.

Additional information. Metronidazole is stable in air, but darkens on exposure to light.

Requirements

Definition. Metronidazole contains not less than 99.0% and not more than 101.0% of $C_6H_9N_3O_3$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from metronidazole RS or with the *reference spectrum* of metronidazole.

B. To 10 mg add 10 mg of zinc R powder, 1 ml of water, and 0.25 ml of hydrochloric acid (~250 g/l) TS and heat in a water-bath for 5 minutes; cool in ice, add 0.5 ml of sodium nitrite (100 g/l) TS, and remove the excess nitrite with sufficient sulfamic acid (50 g/l) TS. Add 0.5 ml of the resulting solution to a mixture of 0.5 ml of 2-naphthol TS1 and 2 ml of sodium hydroxide (~80 g/l) TS; an orange-red colour is produced.

Melting range. 159–163 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of diethylamine R as the mobile

phase. Apply separately to the plate 5 µl of each of 2 solutions in acetone R containing (A) 20 mg of the test substance per ml (warm slightly if necessary to dissolve the substance) and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 3 drops of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

Additional requirements for Metronidazole for parenteral use

Complies with the monograph for "Parenteral preparations".

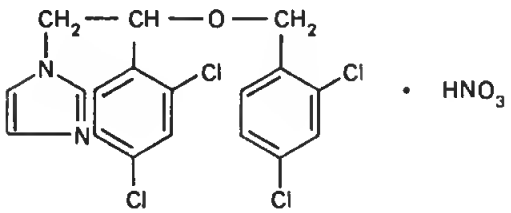
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.35 IU of endotoxin RS per mg.

MICONAZOLI NITRAS
MICONAZOLE NITRATE

Molecular formula. $C_{18}H_{14}Cl_4N_2O,HNO_3$

Relative molecular mass. 479.2

Graphic formula.



Chemical name. 1-[2,4-Dichloro-β-[(2,4-dichlorobenzyl)oxy]-phenethyl]imidazole mononitrate; 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]-ethyl]-1*H*-imidazole mononitrate; CAS Reg. No. 22832-87-7.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water and ether R; soluble in 140 parts of ethanol (~750 g/l) TS.

Category. Antifungal drug.

Storage. Miconazole nitrate should be kept in a well-closed container, protected from light.

Additional information. Miconazole nitrate melts at about 182 °C with decomposition.

Requirements

Definition. Miconazole nitrate contains not less than 98.5% and not more than 101.5% of $C_{18}H_{14}Cl_4N_2O,HNO_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from miconazole nitrate RS or with the *reference spectrum* of miconazole nitrate.

B. The absorption spectrum of a 0.40 mg/ml solution in a mixture of 9 volumes of methanol R and 1 volume of hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits maxima at about 264 nm, 272 nm, and 280 nm; the absorbances of a 1-cm layer at these wavelengths are about 0.40, 0.58, and 0.48, respectively.

C. Shake 10 mg with 5 ml of water and cool in an ice-bath. Keeping the suspension cool throughout, add 0.4 ml of potassium chloride (100 g/l) TS, 0.1 ml of diphenylamine/sulfuric acid TS, and, drop by drop with shaking, 5 ml of sulfuric acid (~1760 g/l) TS; an intense blue colour is produced.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 60 volumes of hexane R, 30 volumes of chloroform R, 10 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply sepa-

rately to the plate 50 µl of each of 2 solutions in a mixture of equal volumes of chloroform R and methanol R containing (A) 10 mg of the test substance per ml and (B) 25 µg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with iodine/chloroform TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

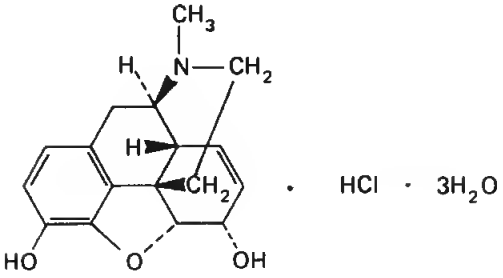
Assay. Dissolve about 0.35 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 47.92 mg of $C_{18}H_{14}Cl_4N_2O,HNO_3$.

MORPHINI HYDROCHLORIDUM
MORPHINE HYDROCHLORIDE

Molecular formula. $C_{17}H_{19}NO_3,HCl,3H_2O$

Relative molecular mass. 375.9

Graphic formula.



Chemical name. 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol hydrochloride (1:1) (salt) trihydrate; CAS Reg. No. 6055-06-7.

Description. Colourless, needle-like crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 25 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Analgesic.

Storage. Morphine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Morphine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Morphine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{17}H_{19}NO_3$,HCl, calculated with reference to the dried substance.

Identity tests

A. To 5 ml of a 1 mg/ml solution add 3 drops of a freshly prepared potassium ferricyanide (10 g/l) TS and 1 drop of ferric chloride (25 g/l) TS; a bluish green colour is produced.

B. To 5 ml of a 1 mg/ml solution add 1 ml of hydrogen peroxide (~60 g/l) TS, 1 ml of ammonia (~100 g/l) TS, and 1 drop of copper (II) sulfate (80 g/l) TS; a transient red colour is produced.

C. To about 1 mg add 0.5 ml of sulfuric acid (~1760 g/l) TS containing 1 drop of formaldehyde TS; a purple colour is produced, which changes quickly to violet.

D. Dissolve a few mg in 5 ml of water, add 1 drop of hydrochloric acid (~70 g/l) TS and 1 ml of potassium iodobismuthate TS2; an orange or orange-red precipitate is produced immediately.

E. A 20 mg/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 20 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -109$ to -115 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 115 mg/g and not more than 150 mg/g.

Acidity. Dissolve 0.2 g in 10 ml of carbon-dioxide-free water R, and titrate with sodium hydroxide (0.02 mol/l) VS, using methyl red/ethanol TS as indicator; not more than 0.2 ml is required to obtain the midpoint of the indicator (orange).

Meconate. Dissolve 0.2 g in 5 ml of water, add 5 ml of hydrochloric acid (~70 g/l) TS and a few drops of ferric chloride (25 g/l) TS; no red colour is produced.

Related alkaloids. Transfer about 0.5 g, accurately weighed, to a separator, add 15 ml of water, 2 ml of sodium hydroxide (10 g/l) TS, and 10 ml of chloroform R. Shake, allow to separate and transfer the chloroform layer to another separator. Repeat the extraction with further quantities of chloroform R, each of 10 ml. Wash the combined chloroform solutions with 4 ml of sodium hydroxide (10 g/l) TS and then twice with water, using 5 ml each time. Separate the chloroform layer and evaporate it carefully to dryness on a water-bath. Add to the residue thus obtained 10 ml of sulfuric acid (0.01 mol/l) VS, heat until dissolved, cool, add 1 drop of methyl red/ethanol TS, and titrate the excess acid with sodium hydroxide (0.02 mol/l) VS; not less than 8.75 ml is required to obtain the midpoint of the indicator (orange).

Noscapine. Dissolve 0.05 g in 2 ml of sulfuric acid (~1760 g/l) TS and heat the solution on a water-bath; no violet colour is produced.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 32.18 mg of $C_{17}H_{19}NO_3$,HCl.

Additional requirement for Morphine hydrochloride for parenteral use

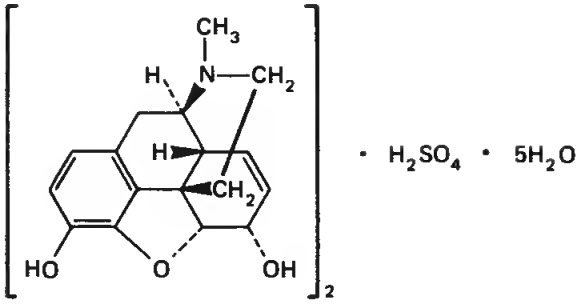
Complies with the monograph for "Parenteral preparations".

MORPHINI SULFAS
MORPHINE SULFATE

Molecular formula. $(C_{17}H_{19}NO_3)_2$,H_2SO_4,$5H_2O$

Relative molecular mass. 758.8

Graphic formula.



Chemical name. 7,8-Didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulfate (2:1) (salt) pentahydrate; CAS Reg. No. 6211-15-0.

Description. White, feathery needles of a white, crystalline powder or cubical masses; odourless.

Solubility. Soluble in 20 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Analgesic.

Storage. Morphine sulfate should be kept in a tightly closed container, protected from light.

Additional information. Morphine sulfate loses water of hydration on exposure to air and darkens in colour on prolonged exposure to light.

Requirements

Definition. Morphine sulfate contains not less than 98.0% and not more than 101.0% of $(C_{17}H_{19}NO_3)_2,H_2SO_4$, calculated with reference to the dried substance.

Identity tests

A. To 5 ml of a 1 mg/ml solution add 3 drops of a freshly prepared potassium ferricyanide (10 g/l) TS and 1 drop of ferric chloride (25 g/l) TS; a bluish green colour is produced.

B. To 5 ml of a 1 mg/ml solution add 1 ml of hydrogen peroxide (~60 g/l) TS, 1 ml of ammonia (~100 g/l) TS and 1 drop of copper (II) sulfate (80 g/l) TS; a transient red colour is produced.

C. To about 1 mg add 0.5 ml of sulfuric acid (~1760 g/l) TS containing 1 drop of formaldehyde TS; a purple colour is produced, which changes quickly to violet.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 20 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -106$ to -110 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 145 °C for 1 hour; it loses not less than 90 mg/g and not more than 120 mg/g.

Acidity. Dissolve 0.2 g in 10 ml of carbon-dioxide-free water R, and titrate with sodium hydroxide (0.02 mol/l) VS, using methyl red/ethanol TS as indicator; not more than 0.2 ml is required to obtain the midpoint of the indicator (orange).

Meconate. Dissolve 0.2 g in 5 ml of water, add 5 ml of hydrochloric acid (~70 g/l) TS and a few drops of ferric chloride (25 g/l) TS; no red colour is produced.

Related alkaloids. Transfer about 0.5 g, accurately weighed, to a separator, add 15 ml of water, 2 ml of sodium hydroxide (10 g/l) TS, and 10 ml of chloroform R. Shake, allow to separate, and transfer the chloroform layer to another separator. Repeat the extraction with further quantities of chloroform R, each of 10 ml. Wash the combined chloroform solutions with 4 ml of sodium hydroxide (10 g/l) TS and twice with water, using 5 ml each time. Separate the chloroform layer and evaporate it carefully to dryness on a water-bath. Add to the residue thus obtained 10 ml of sulfuric acid (0.01 mol/l) VS, heat until dissolved, cool, add 1 drop of methyl red/ethanol TS, and titrate the excess acid with sodium hydroxide (0.02 mol/l) VS; not less than 8.75 ml is required to obtain the midpoint of the indicator (orange).

Noscapine. Dissolve 0.05 g in 2 ml of sulfuric acid (~1760 g/l) TS and heat the solution on a water-bath; no violet colour is produced.

Assay. Dissolve about 0.6 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 66.88 mg of $(C_{17}H_{19}NO_3)_2,H_2SO_4$.

Additional requirements for Morphine sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 14.29 IU of endotoxin RS per mg.

NALOXONI HYDROCHLORIDUM

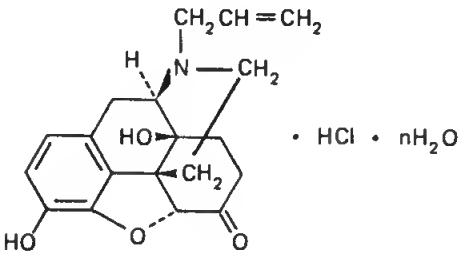
NALOXONE HYDROCHLORIDE

Naloxone hydrochloride, anhydrous
Naloxone hydrochloride, dihydrate

Molecular formula. $C_{19}H_{21}NO_4,HCl$ (anhydrous); $C_{19}H_{21}NO_4,HCl,2H_2O$ (dihydrate).

Relative molecular mass. 363.8 (anhydrous); 399.9 (dihydrate).

Graphic formula.



n = 0 (anhydrous)
n = 2 (dihydrate)

Chemical name. (–)-17-Allyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride; 4,5*a*-epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one hydrochloride; (–)-12-allyl-7,7a,8,9-tetrahydro-3,7a-dihydroxy-4a*H*-8,9c-imino-ethanophenanthro[4,5-*bcd*]furan-5(6*H*)-one hydrochloride; CAS Reg. No. 357-08-4 (anhydrous).

(–)-17-Allyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride dihydrate; 4,5α-epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one hydrochloride dihydrate; (–)-12-allyl-7,7a,8,9-tetrahydro-3,7a-dihydroxy-4a*H*-8,9c-imino-ethanophenanthro[4,5-*bcd*]furan-5(6*H*)-one hydrochloride dihydrate; CAS Reg. No. 51481-60-8 (dihydrate).

Description. A white or almost white powder.

Solubility. Soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Narcotic antagonist.

Storage. Naloxone hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation on the container of Naloxone hydrochloride should state whether the substance is in the anhydrous form or is the dihydrate.

Additional information. Even in the absence of light, Naloxone hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. It melts at about 177 °C.

Requirements

Definition. Naloxone hydrochloride contains not less than 98.0% and not more than 102.0% of $C_{19}H_{21}NO_4,HCl$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from naloxone hydrochloride RS or with the *reference spectrum* of naloxone hydrochloride.

B. Dissolve 0.05 g in 5 ml of hydrochloric acid (0.1 mol/l) VS and add 0.3 ml of ferric chloride (25 g/l) TS; a purplish blue colour is produced.

C. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 25 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -170$ to -181 °.

Loss on drying. Dry to constant weight at 105 °C; anhydrous Naloxone hydro-chloride loses not more than 5.0 mg/g. Naloxone hydrochloride dihydrate loses not more than 110 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance, and as the mobile phase prepare the following solution: shake 100 ml of 1-butanol R with 60 ml of ammonia (~17 g/l) TS, discard the lower layer, and mix 95 volumes of the upper layer with 5 volumes of methanol R. Dry the plate in a current of air. Apply separately to the plate 5 µl of each of the two following solutions. For solution (A) dissolve 40 mg of Naloxone hydrochloride in 2 ml of water and dilute to 5 ml with methanol R. For solution (B) dilute 0.5 ml of solution A to 100 ml with methanol R. Develop the chromatogram protected from light. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with ferric chloride/potassium ferricyanide TS, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Disregard any spot remaining at the point of application.

Chlorides content. Dissolve about 0.3 g, accurately weighed, in 50 ml of methanol R. Add 5 ml of glacial acetic acid R and 0.1 ml of eosin Y (5 g/l) TS, and titrate with silver nitrate (0.1 mol/l) VS until a pink colour is produced. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 3.545 mg of Cl; the content of chlorides is not less than 95.4 mg/g and not more than 99.4 mg/g, calculated with reference to the dried substance.

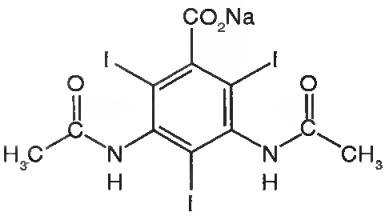
Assay. Dissolve about 0.3 g, accurately weighed, in 40 ml of glacial acetic acid R1, add 10 ml of acetic anhydride R, 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.38 mg of $C_{19}H_{21}NO_4,HCl$.

Additional requirements for Naloxone hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 500 IU of endotoxin RS per mg.

NATRII AMIDOTRIZOAS
SODIUM AMIDOTRIZOATE



$C_{11}H_8I_3N_2NaO_4$

Relative molecular mass. 635.9

Chemical name. Monosodium 3,5-diacetamido-2,4,6-triiodobenzoate; monosodium 3,5-bis(acetylamino)-2,4,6-triiodobenzoate; CAS Reg. No. 737-31-5.

Other names. Sodium diatrizoate; diatrizoate sodium.

Description. A white powder; odourless or almost odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Radiocontrast medium.

Storage. Sodium amidotrizoate should be kept in a well-closed container, protected from light.

Requirements

Sodium amidotrizoate contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{11}H_8I_3N_2NaO_4$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and E or tests B, C, D, and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sodium amidotrizoate RS or with the *reference spectrum* of sodium amidotrizoate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and 20 volumes of chloroform R, 10 volumes of methanol R, and 2 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of two solutions in a mixture of 80 mg of sodium hydroxide R in 100 ml of methanol R containing (A) 1 mg of Sodium amidotrizoate per ml and (B) 1 mg of sodium amidotrizoate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Place 20 mg into a flask, add 5 ml of sodium hydroxide (~80 g/l) TS, and boil gently under a reflux condenser for 10 minutes. Cool, add 5 ml of hydrochloric acid (~70 g/l) TS, and cool in ice for 5 minutes. Add 4 ml of sodium nitrite (10 g/l) TS, cool in ice for 5 minutes, add 0.3 g of sulfamic acid R, swirl gently until effervescence ceases, and add 2 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS; an orange-red colour is produced.

D. Heat 0.1 g in a suitable crucible; violet vapours are evolved.

E. When tested for Sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Iodides. Dissolve 0.8 g in 10 ml of water, add drop by drop nitric acid (~130 g/l) TS until complete precipitation is obtained, then add an excess of 3 ml. Filter, and wash the precipitate with 5 ml of water; to the filtrate add 1 ml of hydrogen peroxide (~330 g/l) TS and 1 ml of chloroform R, and shake. To serve as a reference solution, treat similarly 2 ml of iodide standard (20 µg I/ml) TS with 3 ml of nitric acid (~130 g/l) TS and sufficient water to equal the volume of the solution to be tested. Any red-violet colour in the chloroform layer is not darker than that obtained from the reference solution.

Clarity and colour of solution. A solution of 0.2 g in 10 ml of water is clear and colourless.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.4 g; the water content is not more than 100 mg/g.

pH value. pH of a 0.50 g/ml solution, 7.5–9.5.

Primary aromatic amines. Place about 1 g, accurately weighed, in a 50-ml volumetric flask, and add 5 ml of water, 10 ml of sodium hydroxide (0.1 mol/l) VS, and 25 ml of dimethyl sulfoxide R. Stopper the flask, mix the contents by gentle swirling, and allow to stand in an ice-bath, protected from light, for 5 minutes. Keeping the flask in the ice-bath add slowly 2 ml of hydrochloric acid (~250 g/l) TS, mix, and allow to stand for 5 minutes. Add 1.5 ml of sodium nitrite (35 g/l) TS, mix, and allow to stand for 5 minutes. Add 2 ml of sulfamic acid (50 g/l) TS, mix, and again allow to stand for 5 minutes. Add 2 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride/1-propanol TS and mix. Remove the flask from the ice-bath and allow to stand in water at 22–25 °C for 10 minutes with occasional gentle shaking. Dilute to 50 ml with dimethyl sulfoxide R and mix.

Within 5 minutes after the addition of the last reagent measure the absorbance at about 470 nm, against a solvent cell containing the reagents prepared in a similar manner. The absorbance does not exceed 0.40.

Assay. To about 0.3 g, accurately weighed, add 30 ml of sodium hydroxide (50 g/l) TS and 0.5 g of zinc R powder. Boil under a reflux condenser for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 ml of water, and filter. Thoroughly rinse the flask and add the rinse liquid to the filter. To the filtrate add 5 ml of glacial acetic acid R and 1 ml of tetrabromophenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l) VS until the yellow precipitate formed just turns green.

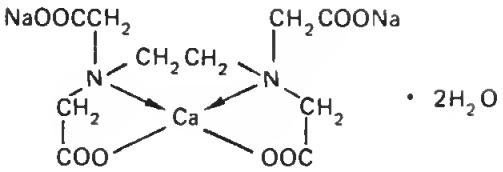
Each ml of silver nitrate (0.05 mol/l) VS is equivalent to 10.60 mg of $C_{11}H_8I_3N_2NaO_4$.

NATRII CALCII EDETAS
SODIUM CALCIUM EDETATE

Molecular formula. $C_{10}H_{12}CaN_2Na_2O_8,2H_2O$

Relative molecular mass. 410.3

Graphic formula.



Chemical name. Disodium [(ethylenedinitrilo)tetraacetato]calciate(2-) dihydrate; (*OC*-6-21)-disodium [[*N*,*N*′-1,2-ethanediylbis[*N*-(carboxymethyl)glycinato]](4-)-*N*,*N*′,*O*,*O*′,O^{N},$O^{N'}$]calciate(2-) dihydrate; calcium chelate of the disodium salt of ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid dihydrate; CAS Reg. No. 6766-87-6 (dihydrate).

Description. A white or creamy white powder; almost odourless.

Solubility. Soluble in 2 parts of water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antidote, chelating agent for metals, mainly lead.

Storage. Sodium calcium edetate should be kept in a tightly closed container.

Additional information. Even in the absence of light, Sodium calcium edetate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Sodium calcium edetate contains not less than 97.0% and not more than 102.0% of $C_{10}H_{12}CaN_2Na_2O_8$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 2.0 g in 25 ml of water, add 2.0 ml of lead nitrate (100 g/l) TS, shake and add 6 ml of potassium iodide (80 g/l) TS; no yellow precipitate can be observed (keep the solution for test C).

B. To 0.15 ml of ferric chloride (25 g/l) TS add 0.15 ml of ammonium thiocyanate (75 g/l) TS; to this deep red-coloured solution add about 0.05 g of the test substance; the deep red colour changes to yellow.

C. To the solution prepared in test A, add ammonia (~100 g/l) TS, drop by drop, until an alkaline reaction is obtained with pH-indicator paper R. Add 5 ml of ammonium oxalate (25 g/l) TS; a white precipitate is produced (distinction from disodium edetate).

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, ignite a small quantity and dissolve the residue in acetic acid (~60 g/l) TS.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Cyanide. Dissolve 1.0 g in 50 ml of water, add 0.1 g of potassium iodide R, 4 ml of ammonia (~100 g/l) TS, and 0.10 ml of silver nitrate (0.01 mol/l) VS, and allow to stand for 1 minute; the opalescence produced is not less than that of a standard solution containing 1 ml of nitric acid (~130 g/l) TS, 1.0 ml of hydrochloric acid (0.0001 mol/l) VS, 9 ml of water, and 0.10 ml of silver nitrate (0.1 mol/l) VS, this solution having also been allowed to stand for 1 minute.

Disodium edetate. Dissolve 5.0 g in 20 ml of water, add 10 ml of ammonia buffer TS2 and 0.2 g of mordant black 11 indicator mixture R, and titrate with magnesium chloride (0.1 mol/l) VS. Not more than 1.5 ml of magnesium chloride (0.1 mol/l) VS is required.

Iron. Ignite 0.5 g and dissolve the residue in 40 ml of water. Treat the solution as described under 2.2.4 Limit test for iron; not more than 80 µg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not more than 130 mg/g.

pH value. pH of a 0.20 g/ml solution in carbon-dioxide-free water R, 6.5–8.0.

Assay. Dissolve about 0.5 g, accurately weighed, in 90 ml of water, add 7 g of methenamine R and 5 ml of hydrochloric acid (~70 g/l) TS. Titrate with lead nitrate (0.05 mol/l) VS, using xylenol orange indicator mixture R. Each ml of lead nitrate (0.05 mol/l) VS is equivalent to 18.71 mg of $C_{10}H_{12}CaN_2Na_2O_8$.

Additional requirements for Sodium calcium edetate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.2 IU of endotoxin RS per mg of sodium edetate.

NATRII CHLORIDUM
SODIUM CHLORIDE

Molecular formula. NaCl

Relative molecular mass. 58.44

Chemical name. Sodium chloride; CAS Reg. No. 7647-14-5.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Category. Ionic equilibration agent.

Storage. Sodium chloride should be kept in a well-closed container.

Additional information. Sodium chloride has a saline taste.

Requirements

Definition. Sodium chloride contains not less than 99.0% and not more than 100.5% of NaCl, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Arsenic. Use a solution of 2.5 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; not more than 4 µg/g.

Barium. Dissolve 4 g in 20 ml of water, filter if necessary, divide the solution into 2 portions and place them in two separate matched tubes. To 1 portion add 2 ml of sulfuric acid (~100 g/l) TS and to the other, 2 ml of water; the solutions remain equally clear for not less than 30 minutes when viewed down the vertical axis of the tube in diffused light against a black background.

Calcium and magnesium. To 20 ml of a 10 mg/ml solution add 2 ml each of ammonia (~100 g/l) TS, ammonium oxalate (25 g/l) TS, and disodium hydrogen phosphate (40 g/l) TS; no turbidity is produced within 5 minutes.

Iodides and bromides. Digest 2 g of finely powdered test substance for 3 hours with 25 ml of warm ethanol (~750 g/l) TS, cool, and filter. Evaporate the filtrate to dryness, dissolve the residue in 5 ml of water, add 1 ml of chloroform R, and cautiously add, drop by drop, with constant agitation, 5 drops of chlorine TS, previously diluted with twice their volume of water; the chloroform does not acquire a violet, yellow or orange colour.

Iron and sodium ferrocyanides. To 0.5 g add 5 drops of sulfuric acid (~100 g/l) TS and carefully heat until dry. Dissolve the residue in 6 ml of water, add 1 ml of hydrochloric acid (~70 g/l) TS, 1 drop of hydrogen peroxide (~60 g/l) TS, and 2 ml of ammonium thiocyanate (~75 g/l) TS; this constitutes solution A. Prepare in a similar manner solution B using 0.40 ml of iron standard FeTS instead of the residue obtained from the test substance. Solution A is not more intensely coloured than solution B when compared as described under 1.11 Colour of liquids (16 µg/g of total Fe).

Sulfates. Dissolve 1.7 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.3 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Loss on drying. Dry to constant weight at 130 °C; it loses not more than 10 mg/g.

Acidity or alkalinity. Dissolve 2 g in 20 ml of carbon-dioxide-free water R and add 2 drops of bromothymol blue/ethanol TS; not more than 0.1 ml of sodium hydroxide (0.02 mol/l) VS or 0.2 ml of hydrochloric acid (0.02 mol/l) VS is required to attain the midpoint of the indicator (green).

Assay. Dissolve about 0.25 g, accurately weighed, in 50 ml of water and titrate with silver nitrate (0.1 mol/l) VS, using potassium chromate (100 g/l) TS as indicator. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 5.844 mg of NaCl.

Additional requirements for Sodium chloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 5.0 IU of endotoxin RS per g.

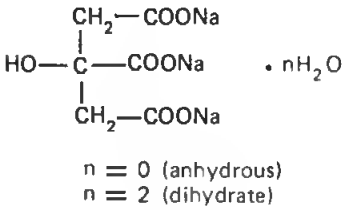
NATRII CITRAS
SODIUM CITRATE

Sodium citrate, anhydrous
Sodium citrate, dihydrate

Molecular formula. $C_6H_5Na_3O_7$ (anhydrous); $C_6H_5Na_3O_7,2H_2O$ (dihydrate).

Relative molecular mass. 258.1 (anhydrous); 294.1 (dihydrate).

Graphic formula.



Chemical name. Trisodium citrate; trisodium 2-hydroxy-1,2,3-propanetricarboxylate; CAS Reg. No. 68-04-2 (anhydrous).

Trisodium citrate dihydrate; trisodium 2-hydroxy-1,2,3-propanetricarboxylate dihydrate; CAS Reg. No. 6132-04-3 (dihydrate).

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water and very soluble in boiling water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Systemic alkalinizing agent; component of oral rehydration salt mixtures.

Storage. Sodium citrate should be kept in a tightly closed container.

Labelling. The designation on the container of Sodium citrate should state whether the substance is the dihydrate or is in the anhydrous form.

Additional information. Sodium citrate is slightly deliquescent in moist air.

Requirements

Definition. Sodium citrate contains not less than 99.0% and not more than 101.0% of $C_6H_5Na_3O_7$, calculated with reference to the anhydrous substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of citrates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Oxalates. Dissolve 0.5 g in 4 ml of water, add 3 ml of hydrochloric acid (~420 g/l) TS and 1 g of granulated zinc R, and heat on a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of phenylhydrazine hydrochloride (10 g/l) TS, and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid (~420 g/l) TS, followed by 0.25 ml of potassium ferricyanide (50 g/l) TS. Shake and allow to stand for 30 minutes; any pink colour produced is not more intense than that of a similarly treated solution containing 4 ml of oxalic acid (0.05 g/l) TS.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. For the anhydrous form use about 1 g of the substance; the water content is not more than 10 mg/g. For the dihydrate use about 0.3 g of the substance; the water content is not less than 0.10 g/g and not more than 0.13 g/g.

Acidity or alkalinity. Dissolve 1 g in 10 ml of carbon-dioxide-free water R and add 0.1 ml of phenolphthalein/ethanol TS; not more than 0.2 ml of hydrochloric acid (0.1 mol/l) VS or 0.2 ml of sodium hydroxide (0.1 mol/l) VS is required to change the colour of the solution.

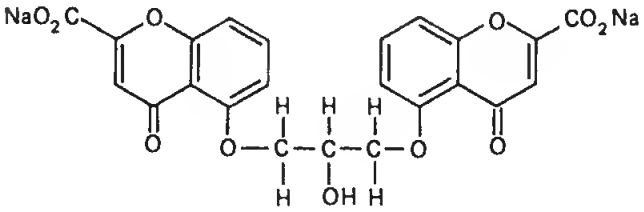
Assay. Dissolve about 0.15 g, accurately weighed, in 20 ml of glacial acetic acid R1, heat to about 50 °C, allow to cool to room temperature, add 0.25 ml of 1-naphtholbenzein/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS until a green colour is obtained as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 8.603 mg of $C_6H_5Na_3O_7$.

NATRII CROMOGLICAS
SODIUM CROMOGLICATE

Molecular formula. $C_{23}H_{14}Na_2O_{11}$

Relative molecular mass. 512.3

Graphic formula.



Chemical name. Disodium 5,5′-[(2-hydroxytrimethylene)dioxy]bis[4-oxo-4*H*-1-benzopyran-2-carboxylate]; disodium 5,5′-[(2-hydroxy-1,3-propanediyl) bis(oxy)]-bis[4-oxo-4*H*-1-benzopyran-2-carboxylate]; CAS Reg. No. 15826-37-6.

Description. A white, crystalline powder; odourless.

Solubility. Freely soluble in water; slightly soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiasthmatic drug.

Storage. Sodium cromoglicate should be kept in a tightly closed container, protected from light.

Additional information. Sodium cromoglicate is hygroscopic.

Requirements

Definition. Sodium cromoglicate contains not less than 98.0% and not more than 101.0% of $C_{23}H_{14}Na_2O_{11}$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the

spectrum obtained from sodium cromoglicate RS or with the reference spectrum of sodium cromoglicate.

B. See the test described below under "Related substances". The spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Dissolve 5 mg in 0.5 ml of methanol R and add 3 ml of 4-aminoantipyrine TS1, and allow to stand for 5 minutes; an intense yellow colour is produced.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Oxalates. Dissolve 0.10 g in 20 ml of water, add 5.0 ml of iron salicylate TS and sufficient water to produce 50 ml; the absorbance at about 480 nm is not less than that of a solution containing 0.35 mg of oxalic acid R prepared in a similar manner.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 100 mg/g.

Acidity or alkalinity. Dissolve 1.0 g in 25 ml of carbon-dioxide-free water R and add 0.1 ml of bromothymol blue/ethanol TS; not more than 0.25 ml of sodium hydroxide (0.1 mol/l) VS or 0.25 ml of hydrochloric acid (0.1 mol/l) VS is required to obtain the midpoint of the indicator (green).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance, omitting the heating, and allowing the plate to stand overnight at room temperature. As the mobile phase use a mixture of 9 volumes of chloroform R, 9 volumes of methanol R, and 2 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of 3 solutions in a mixture of 1 volume of acetone R, 4 volumes of tetrahydrofuran R (which has been freed from stabilizer by passage through a column of suitable alumina), and 6 volumes of water, containing (A) 20 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of sodium cromoglicate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, moving ahead of the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.18 g, accurately weighed, in a mixture of 25 ml of propylene glycol R and 5 ml of 2-propanol R, warming slightly, cool and add 30 ml of dioxan R. Titrate with perchloric acid/dioxan (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid/dioxan (0.1 mol/l) VS is equivalent to 25.62 mg of $C_{23}H_{14}Na_2O_{11}$.

NATRII FLUORIDUM
SODIUM FLUORIDE

Molecular formula. NaF

Relative molecular mass. 41.99

Chemical name. Sodium fluoride; CAS Reg. No. 7681-49-4.

Description. A white powder; odourless.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Mineral salt.

Storage. Sodium fluoride should be kept in a well-closed container.

Requirements

Definition. Sodium fluoride contains not less than 98.0% and not more than 101.0% of NaF, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. Place 0.10 g in a lead or platinum crucible, add about 1 ml of sulfuric acid (~1760 g/l) TS, cover the crucible with a piece of clear polished glass, and heat on a water-bath for 15 minutes. Remove the glass cover, rinse with water and wipe dry; the surface of the glass is etched.

C. Add a few mg of the test substance to a mixture of 0.10 ml of freshly prepared sodium alizarinsulfonate (10 g/l) TS and 0.10 ml of zirconyl nitrate TS; a red colour develops, which changes to yellow.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 40 µg/g.

Loss on drying. Dry to constant weight at 130 °C; it loses not more than 10 mg/g.

Acidity or alkalinity. Dissolve 1.0 g in 20 ml of water in a platinum dish, add and dissolve into this solution 3 g of potassium nitrate R, and cool to 0 °C. Add 0.15 ml of phenolphthalein/ethanol TS; not more than 2.0 ml of sodium hydroxide (0.05 mol/l) VS or 1.0 ml of sulfuric acid (0.05 mol/l) VS is required to obtain the midpoint of the indicator (pink). (Keep the solution for the test of fluorosilicates.)

Fluorosilicates. Heat to boiling the solution obtained in the test for acidity or alkalinity and titrate while hot with sodium hydroxide (0.05 mol/l) VS until a permanent pink colour is produced; not more than 1.5 ml of sodium hydroxide (0.05 mol/l) VS are required.

Assay. Dissolve about 30 mg, accurately weighed, in 1.0 ml of water. Add cautiously 15 ml of acetic anhydride R and boil gently for 2 minutes, placing a small funnel on the flask to serve as a reflux condenser. Cool and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 4.199 mg of NaF.

NATRII HYDROGENOCARBONAS
SODIUM HYDROGEN CARBONATE

Molecular formula. $NaHCO_3$

Relative molecular mass. 84.01

Chemical name. Monosodium carbonate; CAS Reg. No. 144-55-8.

Other name. Sodium bicarbonate.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Systemic alkalinizing agent; antacid.

Storage. Sodium hydrogen carbonate should be kept in well-closed containers.

Additional information. Sodium hydrogen carbonate has a saline and slightly alkaline taste. It is stable in air, but slowly decomposes in moist air.

Requirements

Definition. Sodium hydrogen carbonate contains not less than 99.0% and not more than 101.0% of $NaHCO_3$, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. To a small amount add hydrochloric acid (~70 g/l) TS; it effervesces and the gas is colourless. Add a few drops of calcium hydroxide TS; immediately a white precipitate is formed.

C. To a 20 mg/ml solution add at room temperature a few drops of magnesium sulfate (50 g/l) TS; no precipitate is formed. Boil the mixture; a white precipitate is formed.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Ammonium. Heat 1.0 g in a test-tube; the gas evolved does not turn moistened red litmus paper R blue.

Arsenic. Use a solution of *3.3 g* in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Calcium. Boil a 20 mg/ml solution for 5 minutes; the solution is clear or at most very slightly opalescent.

Carbonates. pH of a 50 mg/ml solution in carbon-dioxide-free water R, not more than 8.6.

Chlorides. Dissolve 1.7 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 40 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.15 mg/g.

Sulfates. Dissolve 2.5 g in 40 ml of water, add 1 ml of hydrochloric acid (~250 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Loss on drying. Dry to constant weight over silica gel, desiccant, R at ambient temperature; it loses not more than 2.5 mg/g.

Assay. Dissolve about 0.5 g, accurately weighed, in 50 ml of water and titrate with hydrochloric acid (0.2 mol/l) VS, using 3 drops of methyl orange/ethanol TS as indicator. Each ml of hydrochloric acid (0.2 mol/l) VS is equivalent to 16.80 mg of $NaHCO_3$.

Additional requirements for Sodium hydrogen carbonate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 5.0 IU of endotoxin RS per milliequivalence.

NATRII HYDROXYDUM
SODIUM HYDROXIDE

NaOH

Relative molecular mass. 40.00

Chemical name. Sodium hydroxide; sodium hydroxide (Na(OH)); CAS Reg. No. 1310-73-2.

Description. White or almost white, fused masses, sticks, pellets, or flakes; they are hard and brittle, showing a crystalline fracture.

Solubility. Very soluble in water and ethanol (~750 g/l) TS.

Category. Alkalinizing agent; used in the preparation of sodium lactate solution.

Storage. Sodium hydroxide should be kept in a tightly closed container.

Additional information. *CAUTION*: Sodium hydroxide must be handled with care, avoiding contact with the skin. It is very deliquescent, strongly alkaline, and corrosive. It rapidly absorbs carbon dioxide.

Requirements

Sodium hydroxide contains not less than **97.5%** of total alkali, calculated as NaOH, and not more than the equivalent of **2.5%** of Na_2CO_3.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. A solution is strongly alkaline.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Arsenic. Use a solution of 2.5 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 4 µg/g.

Aluminium, iron, and matter insoluble in hydrochloric acid. Boil 5 g with 70 ml of hydrochloric acid (~70 g/l) TS. Cool, make alkaline with ammonia (~100 g/l) TS, boil, filter, and wash with a mixture of equal volumes of water and ammonium nitrate (50 g/l) TS. Ignite the residue to constant mass; not more than 5 mg.

Potassium. Dissolve 0.25 g in 5 ml of water, acidify with acetic acid (~60 g/l) TS, and add 5 drops of sodium cobaltinitrite (100 g/l) TS; no precipitate is formed.

Chlorides. Dissolve 0.35 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.7 mg/g.

Sulfates. Dissolve 0.4 g in 20 ml of water, add 6 ml of hydrochloric acid (~70 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1.2 mg/g.

Assay. Dissolve about 2 g, accurately weighed, in 80 ml of carbon-dioxide-free water R. Add 0.3 ml of phenolphthalein/ethanol TS and titrate with hydrochloric acid (1 mol/l) VS until the red colour of the indicator disappears. Note the consumption of acid. Add 0.3 ml of methyl orange/ethanol TS to the solution

and continue the titration with hydrochloric acid (1 mol/l) VS until a permanent red colour is obtained.

Each ml of hydrochloric acid (1 mol/l) VS used in the second titration is equivalent to 106.0 mg of Na_2CO_3. Each ml of hydrochloric acid (1 mol/l) VS used in the combined titrations is equivalent to 40.00 mg of total alkali, calculated as NaOH.

NATRII NITRIS
SODIUM NITRITE

Molecular formula. $NaNO_2$

Relative molecular mass. 69.00

Chemical name. Nitrous acid, sodium salt; CAS Reg. No. 7632-00-0.

Description. A white to slightly yellow, granular powder, or white or almost white, opaque, fused masses or sticks; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Vasodilator.

Storage. Sodium nitrite should be kept in a well-closed container, protected from light.

Additional information. Sodium nitrite is deliquescent in air. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Sodium nitrite contains not less than 98.0% and not more than 100.5% of $NaNO_2$, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, prepare a 0.10 g/ml solution acidified with acetic acid (~300 g/l) TS.

B. Dissolve 0.10 g in 1.0 ml of water and add 1.0 ml of ferrous sulfate(~15 g/l) TS; after a few minutes a deep brown colour is produced.

C. Dissolve 0.20g in 1.0ml of water and add 1.0ml of sulfuric acid (~100g/l) TS; brownish red fumes are evolved. Add a few drops of starch/iodide TS; a blue colour is produced.

Heavy metals. Dissolve 1.0g in 10ml of hydrochloric acid (~70g/l) TS, evaporate to dryness on a water-bath and until the odour of hydrochloric acid is no longer perceptible. Proceed with the residue as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20µg/g.

Chlorides. For the preparation of the test solution, boil 2.5g in a mixture of 3ml of water and 2ml of nitric acid (~1000g/l) TS, cool, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.1mg/g.

Loss on drying. Dry at ambient temperature over silica gel, desiccant, R for 4 hours; it loses not more than 5.0mg/g.

Acidity or alkalinity. To a solution of 0.5g in 10ml of water add 0.1ml of sodium hydroxide (0.01mol/l) VS and 0.25ml of phenol red/ethanol TS; a red colour is produced. Add 0.3ml of hydrochloric acid (0.01mol/l) VS; the colour changes to yellow.

Assay. Dissolve about 0.4g, accurately weighed, in sufficient water to produce 100ml. To 10.0ml of this solution add 20.0ml of potassium permanganate (0.02mol/l) VS and 10ml of sulfuric acid (~1760g/l) TS. Allow to stand for 10 minutes, add 0.5g of potassium iodide R, swirl the flask, and titrate the liberated iodine with sodium thiosulfate (0.1mol/l) VS, using starch TS as indicator. Each ml of sodium thiosulfate (0.1mol/l) VS is equivalent to 3.450mg of $NaNO_2$.

Additional requirements for Sodium nitrite for parenteral use

Complies with the monograph for "Parenteral preparations".

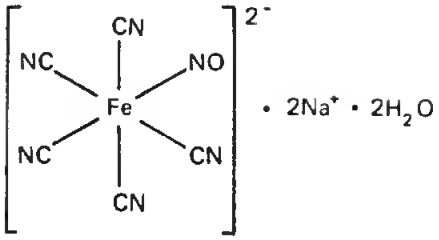
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.33IU of endotoxin RS per mg.

NATRII NITROPRUSSIDUM
SODIUM NITROPRUSSIDE

Molecular formula. $Na_2[Fe(CN)_5NO],2H_2O$

Relative molecular mass. 298.0

Graphic formula.



Chemical name. Disodium pentacyanonitrosylferrate(2-) dihydrate; disodium (*OC*-6-22)-pentakis(cyano-*C*)nitrosylferrate(2-) dihydrate; CAS Reg. No. 13755-38-9 (dihydrate).

Other names. Sodium nitroferricyanide; sodium nitroprussiate.

Description. Reddish brown crystals or powder; odourless or almost odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Category. Antihypertensive drug.

Storage. Sodium nitroprusside should be kept in a tightly closed container, protected from light.

Requirements

Definition. Sodium nitroprusside contains not less than 99.0% and not more than 100.5% of $Na_2[Fe(CN)_5NO]$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 4.0 mg/ml solution, when observed between 350 nm and 600 nm, exhibits a maximum at about 395 nm; the absorbance of a 1-cm layer at this wavelength is about 1.10.

B. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, prepare a 0.10 g/ml solution acidified with acetic acid (~300 g/l) TS.

C. Dissolve 5 mg in 2.0 ml of water, add 0.1 ml of acetone R and 0.5 ml of sodium hydroxide (~80 g/l) TS; an orange colour is produced. Add about 2 ml of acetic acid (~300 g/l) TS; the colour changes to purple.

Chlorides. Dissolve 1.20 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfates. Dissolve 5.0 g in 40 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.1 mg/g.

Ferricyanide. Dissolve 0.50 g in 20 ml of ammonium acetate buffer, pH 4.62, TS, and divide the solution into two equal portions, A and B. To portion B add 1 ml of ferricyanide standard (50 µg/ml) TS, then add to both portions 5 ml of ferrous ammonium sulfate (1 g/l) TS and dilute to 50 ml with water. Prepare a blank solution by dissolving 0.25 g of the substance to be examined in 10 ml of ammonium acetate buffer, pH 4.62, TS, and diluting to 50 ml with water. Allow to stand for 1 hour and measure the absorbance of the solutions at the maximum at about 720 nm. The absorbance of portion A when measured against the blank is not greater than the absorbance of portion B when measured against portion A (0.2 mg/g).

Ferrocyanide. Dissolve 2.0 g in 40 ml of water and divide the solution into two equal portions, A and B. To portion B add 2 ml of ferrocyanide standard (100 µg/ml) TS, then add to both portions 0.2 ml of ferric chloride (50 g/l) TS and dilute to 50 ml with water. Allow to stand for 5 minutes. Prepare a blank solution by dissolving 1.0 g of the substance to be examined in sufficient water to produce 50 ml. Measure the absorbance of the solutions at the maximum at about 695 nm. The absorbance of portion A when measured against the blank is not greater than the absorbance of portion B when measured against portion A (0.2 mg/g).

Insoluble matter. Dissolve 10.0 g in 50 ml of water, heat the solution on a water-bath for 30 minutes, filter, wash the insoluble matter with water, and dry to constant weight at 105 °C; the content of insoluble matter is not more than 0.1 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not less than 90 mg/g and not more than 150 mg/g.

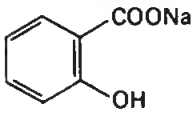
Assay. Dissolve about 0.35 g, accurately weighed, in 100 ml of water. Add 0.1 ml of sulfuric acid (~100 g/l) TS and 20 ml of ethanol (~750 g/l) TS. Titrate with silver nitrate (0.1 mol/l) VS, determining the endpoint potentiometrically using a silver/silver chloride electrode system. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 13.10 mg of $Na_2[Fe(CN)_5NO]$.

NATRII SALICYLAS
SODIUM SALICYLATE

Molecular formula. $C_7H_5NaO_3$

Relative molecular mass. 160.1

Graphic formula.



Chemical name. Sodium 2-hydroxybenzoate; CAS Reg. No. 54-21-7.

Description. Small, colourless crystals or shiny flakes, or a white, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Analgesic; antiphlogistic.

Storage. Sodium salicylate should be kept in a well-closed container, protected from light.

Additional information. Sodium salicylate is discoloured on exposure to light.

Requirements

Definition. Sodium salicylate contains not less than 99.0% and not more than 101.0% of $C_7H_5NaO_3$, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. A 0.05 g/ml solution yields the reaction described under 2.1 General identification tests as characteristic of salicylates.

Heavy metals. For the preparation of the test solution use 2.0 g dissolved in 45 ml of water, add 5 ml of hydrochloric acid (~70 g/l) TS, and filter. Dilute 25 ml of the filtrate to 40 ml with water and mix; determine the heavy metals

content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 1.25 g in a mixture of 5 ml of water and 5 ml of ethanol (~710 g/l) TS. Add 1 ml of nitric acid (~1000 g/l) TS, filter, and proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfates. Dissolve 0.85 g in 20 ml of water, add 1 ml of hydrochloric acid (~250 g/l) TS, and filter. Proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.6 mg/g.

Sulfites and thiosulfates. Dissolve 1.0 g in 20 ml of water, add 1 ml of hydrochloric acid (~250 g/l) TS, and filter. Titrate the filtrate with iodine (0.05 mol/l) VS; not more than 0.15 ml of titrant is required to produce a yellow colour.

Clarity and colour of solution. A freshly prepared solution of 1.0 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Rd1 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Dissolve 2.0 g in 50 ml of carbon-dioxide-free water R and add 10 drops of phenol red/ethanol TS; the solution is yellow. Titrate with sodium hydroxide (0.1 mol/l) VS; not more than 0.2 ml is required to produce a red colour.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each nil of perchloric acid (0.1 mol/l) VS is equivalent to 16.01 mg of $C_7H_5NaO_3$.

NATRII STIBOGLUCONAS
SODIUM STIBOGLUCONATE

Chemical name. D-Gluconic acid cyclic ester with antimonic acid ($H_8Sb_2O_9$) (2 : 1), trisodium salt, nonahydrate; 2,4:2′,4′-*O*-(oxydistibylidyne)bis-D-gluconic acid, *Sb*,*Sb*′-dioxide, trisodium salt, nonahydrate; CAS Reg. No. 16037-91-5.

Description. A colourless, mostly amorphous powder; odourless.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Antileishmaniasis drug.

Storage. Sodium stibogluconate should be kept in a well-closed container.

Requirements

Definition. Sodium stibogluconate is a pentavalent antimony compound of indefinite composition. It has been represented by the formula $C_6H_9Na_2O_9Sb$, but it usually contains less than 2 atoms of sodium for each atom of antimony.

Sodium stibogluconate contains not less than 30.0% and not more than 34.0% of total antimony, calculated with reference to the dried substance.

Identity tests

A. Heat a small quantity of the test substance; it chars without melting. Dissolve the residue in acetic acid (~60 g/l) TS; it yields reaction B, characteristic of sodium as described under 2.1 General identification tests.

B. Dissolve 0.5 g in 10 ml of water and add a few drops of hydrogen sulfide TS; an orange precipitate is produced, which dissolves in sodium hydroxide (~80 g/l) TS.

C. A 10 mg/ml solution is dextrorotatory.

Chlorides. Dissolve 2.5 g in a mixture of 50 ml of water, 2 ml of nitric acid (~130 g/l) TS, and 75 ml of acetate buffer, pH 5.0, TS. Titrate with silver nitrate (0.1 mol/l) VS, determining the endpoint potentiometrically; not more than 3.0 ml of silver nitrate (0.1 mol/l) VS are required.

Loss on drying. Dry to constant weight at 130 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 150 mg/g.

Colour and pH value. Dissolve 0.3 g in 10 ml of water and heat the solution in an autoclave under reduced pressure (about 70 kPa) for 30 minutes; the solution is colourless or almost colourless and has a pH between 5.0 and 5.6.

Trivalent antimony. Dissolve 2.0 g in 30 ml of water, add 15 ml of hydrochloric acid (~250 g/l) TS, and titrate with potassium bromate (0.00833 mol/l) VS using methyl/orange TS as indicator; not more than 1.3 ml of potassium bromate (0.00833 mol/l) VS are required.

Assay. Place about 0.25 g, accurately weighed, in a 300-ml long-necked flask, add 10 ml of nitric acid (~1000 g/l) TS and 5 ml of sulfuric acid (~1760 g/l) TS, and heat cautiously over a small flame, keeping the liquid in motion by rotating the flask; remove the flask from the flame at the onset of the first vigorous reaction until this subsides. Continue to heat until white fumes are evolved and allow the liquid to cool. Add 1 ml of nitric acid (~1000 g/l) TS and again heat until white fumes are evolved. Add 1 g of ammonium sulfate R, again heat to the point of fuming, cool thoroughly, and add 1 g of tartaric acid R and 60 ml of water. To the clear, almost colourless solution add 1 g of potassium iodide R and boil the solution gently for about 5 minutes or until free iodine is expelled, the liquid becoming pure yellow in colour. Cool, add sodium hydroxide (~400 g/l) TS until just alkaline (about 15 ml), cool again, acidify with sulfuric acid (~100 g/l) TS until just acid, add an excess of sodium hydrogen carbonate R, and titrate with iodine (0.02 mol/l) VS, using starch TS as indicator if necessary. Each ml of iodine (0.02 mol/l) VS is equivalent to 2.435 mg of total antimony.

NATRII SULFAS
SODIUM SULFATE

Molecular formula. $Na_2SO_4,10H_2O$

Relative molecular mass. 322.2

Chemical name. Disodium sulfate decahydrate; sulfuric acid disodium salt, decahydrate; CAS Reg. No. 7727-73-3 (decahydrate).

Other name. Glauber's salt.

Description. Colourless crystals or a white powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Laxative.

Storage. Sodium sulfate should be kept in a well-closed container.

Additional information. Sodium sulfate partially dissolves in its own water of crystallization at about 33 °C.

Requirements

Definition. Sodium sulfate contains not less than 99.0% and not more than 100.5% of Na_2SO_4, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 0.05 g/ml solution.

B. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Ammonium salts. Transfer 1 g to a test-tube, add about 0.3 g of potassium hydroxide R and heat the mixture; a moistened red litmus paper R placed in the evolved vapours does not turn blue.

Arsenic. Use a solution of 5 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Calcium. To two separate comparison tubes transfer 0.2 ml of ethanolic calcium standard (100 µg/ml Ca) TS, add 1.5 ml of ammonium oxalate (25 g/l) TS, allow to stand for 1 minute, and then add 1 ml of acetic acid (~120 g/l) TS. To one tube add a solution of the substance to be examined containing 0.5 g in 15 ml of water, and to the second tube add 10 ml of calcium standard (10 µg/ml Ca) TS and 5 ml of water. Observe any opalescence produced after 15 minutes; the opalescence in the first tube is not more intense than that in the second tube (200 µg/g).

Chlorides. Dissolve 1.25 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Iron. Using 1.0 g prepare a solution in 40 ml of water and proceed as described under 2.2.4 Limit test for iron; not more than 40 µg/g.

Magnesium. Dissolve 0.5 g in 10 ml of water, add 1 ml of glycerol R, 0.15 ml of titan yellow TS, 0.2 ml of ammonium oxalate (50 g/l) TS, and 5 ml of sodium hydroxide (~80 g/l) TS, and shake; any pink colour produced is not more intense than that of a similarly treated mixture of 5 ml of magnesium standard (10 µg/ml Mg) TS and 5 ml of water.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Reducing substances. Dissolve 0.5 g in 5 ml of water, add 1 ml of sulfuric acid (~100 g/l) TS and 0.20 ml of potassium permanganate (0.002 mol/l) VS. Allow to stand for 15 minutes; no discoloration is observed.

Loss on drying. Dry at 30 °C for 1 hour and then to constant weight at 130 °C; it loses not less than 0.52 g/g and not more than 0.57 g/g.

Acidity or alkalinity. Dissolve 0.5 g in 10 ml of carbon-dioxide-free water R and add 0.1 ml of bromothymol blue/ethanol TS; not more than 0.5 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS or 0.5 ml of hydrochloric acid (0.01 mol/l) VS is required to obtain the midpoint of the indicator (green).

Assay. Dissolve about 0.25 g, accurately weighed, in 250 ml of water, add 10 ml of hydrochloric acid (~70 g/l) TS, heat to boiling and add a sufficient quantity of barium chloride (50 g/l) TS. Heat on a water-bath for 30 minutes, stirring occasionally. Collect the precipitate, wash, dry and ignite at 600 °C. Each g of residue is equivalent to 0.608 g of Na_2SO_4.

NATRII SULFAS ANHYDRICUS
SODIUM SULFATE, ANHYDROUS

Molecular formula. Na_2SO_4

Relative molecular mass. 142.0

Chemical name. Disodium sulfate; sulfuric acid disodium salt, anhydrous; CAS Reg. No. 7757-82-6 (anhydrous).

Description. A white powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Laxative.

Storage. Anhydrous sodium sulfate should be kept in a well-closed container.

Additional information. Anhydrous sodium sulfate is hygroscopic.

Requirements

Definition. Anhydrous sodium sulfate contains not less than 99.0% and not more than 100.5% of Na_2SO_4, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 45 µg/g.

Ammonium salts. Transfer 0.5 g to a test-tube, add about 0.3 g of potassium hydroxide R and heat the mixture; a moistened red litmus paper R placed in the evolved vapours does not turn blue.

Arsenic. Use a solution of 2.0 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 5 µg/g.

Calcium. To two separate comparison tubes transfer 0.2 ml of ethanolic calcium standard (100 µg/ml Ca) TS, add 1.5 ml of ammonium oxalate (25 g/l) TS, allow to stand for 1 minute, and then add 1 ml of acetic acid (~120 g/l) TS. To one tube add a solution of the substance to be examined containing 0.22 g in 15 ml of water, and to the second tube add 10 ml of calcium standard (10 µg/ml Ca) TS and 5 ml of water. Observe any opalescence produced after 15 minutes; the opalescence in the first tube is not more intense than that in the second tube (450 µg/g).

Chlorides. Dissolve 0.55 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.45 mg/g.

Iron. Using 0.44 g prepare a solution in 40 ml of water and proceed as described under 2.2.4 Limit test for iron; not more than 90 µg/g.

Magnesium. Dissolve 0.22 g in 10 ml of water, add 1 ml of glycerol R, 0.15 ml of titan yellow TS, 0.25 ml of ammonium oxalate (50 g/l) TS, and 5 ml of sodium hydroxide (~80 g/l) TS, and shake; any pink colour produced is not more intense than that of a similarly treated mixture of 5 ml of magnesium standard (10 µg/ml Mg) TS and 5 ml of water.

Clarity and colour of solution. A solution of 0.22 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Reducing substances. Dissolve 0.25 g in 5 ml of water, add 1 ml of sulfuric acid (~100 g/l) TS and 0.20 ml of potassium permanganate (0.002 mol/l) VS. Allow to stand for 15 minutes; no discoloration is observed.

Loss on drying. Dry to constant weight at 130 °C; it loses not more than 50 mg/g.

Acidity or alkalinity. Dissolve 0.22 g in 10 ml of carbon-dioxide-free water R and add 0.1 ml of bromothymol blue/ethanol TS; not more than 0.5 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS or 0.5 ml of hydrochloric acid (0.01 mol/l) VS is required to obtain the midpoint of the indicator (green).

Assay. Dissolve about 0.1 g, accurately weighed, in 250 ml of water, add 10 ml of hydrochloric acid (~70 g/l) TS, heat to boiling, and add a sufficient quantity of barium chloride (50 g/l) TS. Heat on a water-bath for 30 minutes, stirring occasionally. Collect the precipitate, wash, dry and ignite at 600 °C. Each g of residue is equivalent to 0.608 g of Na_2SO_4.

NATRII THIOSULFAS
SODIUM THIOSULFATE

Molecular formula. $Na_2S_2O_3,5H_2O$

Relative molecular mass. 248.2

Chemical name. Disodium thiosulfate pentahydrate; disodium thiosulfate ($Na_2S_2O_3$) pentahydrate; thiosulfuric acid ($H_2S_2O_3$), disodium salt, pentahydrate; CAS Reg. No. 10102-17-7 (pentahydrate).

Description. Transparent, colourless crystals; odourless.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Category. Antidote.

Storage. Sodium thiosulfate should be kept in a tightly closed container.

Additional information. Sodium thiosulfate effloresces in dry air at temperatures exceeding 33 °C and dissolves in its water of crystallization at about 49 °C.

Requirements

Definition. Sodium thiosulfate contains not less than 99.0% and not more than 101.0% of $Na_2S_2O_3,5H_2O$.

Identity tests

A. Dissolve 1.0 g in 10 ml of water (use this solution also for tests B and C). To 2 ml add 1.0 ml of iodine TS; the colour of the solution is discharged. Add 0.25 ml of barium chloride (50 g/l) TS; the solution remains clear.

B. To 2 ml of the solution prepared in test A add 1.0 ml of hydrochloric acid (~70 g/l) TS; an odour of sulfur dioxide is perceptible and a precipitate of sulfur is produced (proceed with caution).

C. To 2 ml of the solution prepared in test A add 2 ml of silver nitrate (0.1 mol/l) VS; a white precipitate is formed, but quickly becomes yellowish, then black.

D. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, prepare a 0.10 g/ml solution.

Heavy metals. Dissolve 1.0 g in 10 ml of water, add slowly 5 ml of hydrochloric acid (~70 g/l) TS, and evaporate to dryness on a water-bath. Add 15 ml of water to the residue, boil gently for 2 minutes and filter. Heat the filtrate to boiling, add sufficient bromine TS1 to the hot filtrate to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the excess bromine. Cool, add 0.05 ml of phenolphthalein/ethanol TS and neutralize with sodium hydroxide (1 mol/l) VS. Adjust the pH to 3–4 with acetic acid (~60 g/l) TS, dilute to 40 ml with water, and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 1.20 g in a mixture of 20 ml of water and 2 ml of nitric acid (~130 g/l) TS, boil gently for 3–4 minutes, cool, filter, and proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Sulfates and sulfites. Dissolve 0.25 g in 10 ml of water, add 5 ml of iodine TS, and gradually more iodine TS, drop by drop, until a very faint persistent yellow colour is produced. Proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 2 mg/g.

Sulfides. Dissolve 1 g in 10 ml of water and add 0.05 ml of sodium nitroprusside (45 g/l) TS; the solution does not become violet.

Clarity and colour. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 6.0–8.4.

Assay. Dissolve about 0.5 g, accurately weighed, in 25 ml of water and titrate with iodine (0.05 mol/l) VS using starch TS as indicator, added towards the end of the titration. Each ml of iodine (0.05 mol/l) VS is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

Additional requirements for Sodium thiosulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

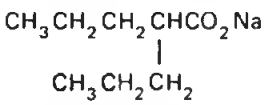
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.03 IU of endotoxin RS per mg.

NATRII VALPROAS
SODIUM VALPROATE

Molecular formula. $C_8H_{15}NaO_2$

Relative molecular mass. 166.2

Graphic formula.



Chemical name. Sodium 2-propylvalerate; sodium 2-propylpentanoate; CAS Reg. No. 1069-66-5.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS.

Category. Antiepileptic drug.

Storage. Sodium valproate should be kept in a well-closed container.

Additional information. Sodium valproate is deliquescent.

Requirements

Definition. Sodium valproate contains not less than 98.0% and not more than 101.0% of $C_8H_{15}NaO_2$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.5 g in 5 ml of water, add 5 ml of chloroform R and 1 ml of hydrochloric acid (~70 g/l) TS, shake vigorously for 1 minute, allow to separate, dry the lower layer with anhydrous sodium sulfate R, filter, and evaporate to dryness. Carry out the examination of a thin film of the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from valproic acid RS or with the *reference spectrum* of valproic acid.

B. Dissolve 0.5 g in 5 ml of water and add 1 ml of cobalt(II) nitrate (100 g/l) TS; a purple precipitate is produced, which is soluble in carbon tetrachloride R.

C. A 20 mg/ml solution yields reaction B, described under 2.1 General identification tests as characteristic of sodium.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Arsenic. Use a solution of 5.0 g in 35 ml of water and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 2 µg/g.

Chlorides. Dissolve 1.20 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.2 mg/g.

Iron. Using 0.2 g prepare a solution in 40 ml of water and proceed as described under 2.2.4 Limit test for iron; not more than 50 µg/g.

Sulfates. Dissolve 2.5 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Clarity and colour of solution. The opalescence of a solution of 2.0 g in 10 ml of carbon-dioxide-free water R is not more intense than that of opalescence standard TS2 and the solution is colourless.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Acidity or alkalinity. Dissolve 2.0 g in 20 ml of carbon-dioxide-free water R and add 0.1 ml of phenolphthalein/ethanol TS; not more than 1.5 ml of sodium

hydroxide (0.1 mol/l) VS or 1.5 ml of hydrochloric acid (0.1 mol/l) VS is required to obtain the midpoint of the indicator (pink).

Related substances. Carry out the test as described under 1.14.5 Gas chromatography using 3 solutions:

Solution 1. 0.20 mg of octanoic acid R (internal standard) per ml of dichloromethane R.

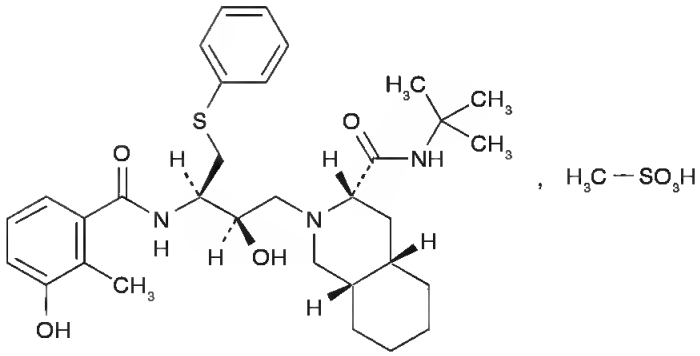
Solution 2. Dissolve 0.50 g of the substance being examined in 10 ml of water, acidify with sulfuric acid (~190 g/l) TS, and shake with 3 quantities, each of 20 ml, of dichloromethane R. Wash the combined dichloromethane extracts with 10 ml of water, shake with anhydrous sodium sulfate R, filter and evaporate the filtrate at a temperature not exceeding 30 °C to a volume of about 10 ml, using a rotary evaporator.

Solution 3. Dissolve 0.50 g of the substance being examined in 10 ml of a mixture of 2.0 mg of octanoic acid R in 10 ml of sodium hydroxide (0.1 mol/l) VS, acidify with sulfuric acid (~190 g/l) TS, and shake with 3 quantities, each of 20 ml, of dichloromethane R. Wash the combined dichloromethane extracts with 10 ml of water, shake with anhydrous sodium sulfate R, filter and evaporate the filtrate at a temperature not exceeding 30 °C to a volume of about 10 ml, using a rotary evaporator.

For the procedure use a glass column, 1.5 m long and 0.4 cm in internal diameter, packed with an adequate quantity of an adsorbent composed of 15 g of a phase consisting of an ester of macrogol 20 M and terephthalic acid, together with 1 g of phosphoric acid (~1440 g/l) TS supported on 84 g of acid-washed, silanized diatomaceous support R (150–180 µm). Maintain the column at 170 °C, use nitrogen R as the carrier gas and a flame ionization detector. In the chromatogram obtained with solution 3, the total area of all the peaks, excluding the main peak and those due to the solvent and the internal standard, is not greater than the area of the peak due to the internal standard.

Assay. Dissolve about 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 16.62 mg of $C_8H_{15}NaO_2$.

NELFINAVIRI MESILAS
NELFINAVIR MESILATE



$C_{32}H_{45}N_3O_4S,CH_4O_3S$

Relative molecular mass. 663.9

Chemical name. (3*S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)-2-[(2*R*,3*R*)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylsulfanyl)butyl]decahydroisoquinoline-3-carboxamide methanesulfonate; CAS reg. No. 159989-65-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Nelfinavir mesilate should be kept in a tightly closed container, protected from light.

Additional information. Nelfinavir mesilate is hygroscopic.

Requirements

Definition. Nelfinavir mesilate contains not less than **98.5%** and not more than **101.0%** of $C_{32}H_{45}N_3O_4S,CH_4O_3S$, calculated with reference to the dried substance.

Manufacture. The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Identity tests

- Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with basic potassium permanganate (~5 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 40 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits a maximum at about 253 nm; the specific absorbance ($A^{1\%}_{1\,cm}$) is 124 to 136.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nelfinavir mesilate RS or with the *reference spectrum* of nelfinavir mesilate.

Specific optical rotation. Use a 10.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -105° \text{ to } -125°$.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 100 °C; it loses not more than 30 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of purified water.

Mobile phase B: 41 volumes of acetonitrile R, 31 volumes of methanol R and 28 volumes of phosphate buffer pH 3.4.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate R in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (~105 g/l) TS and dilute it to 1000 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–27	100	0	Isocratic
27–60	100 to 0	0 to 100	Linear gradient
60–75	0	100	Isocratic
75–80	0 to 100	100 to 0	Return to the initial conditions
80–90	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10.0 µg of Nelfinavir mesilate per ml. For solution (3) use 100 µg of methanesulfonic acid R per ml.

For the system suitability test: prepare solution (4) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 225 nm.

Maintain the column at 35 °C.

Inject 20 µl of solution (4). The test is not valid unless the resolution factor between the principal peak (retention time = about 27 minutes) and the peak with a retention time relative to the principal peak of about 0.2 is not less than 15. The test is also not valid unless the resolution factor between the last two peaks out of three peaks, which increase during the decomposition process, is not less than 4.0. The ratio of the retention times of these two peaks relative to the principal peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile R in both mobile phases A and B, or adjust the gradient program.

Inject 20 µl of solution (3).

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than that in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3).

Assay. Dissolve about 0.50 g, accurately weighed, in 50 ml of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Perform a blank determination and make the necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 66.39 mg of $C_{32}H_{45}N_3O_4S.CH_4O_3S$.

NEOMYCINI SULFAS
NEOMYCIN SULFATE

Chemical name. Neomycin sulfate; CAS Reg. No. 1405-10-3.

Description. A white or yellowish white powder; odourless or almost odourless.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Antiinfective drug.

Storage. Neomycin sulfate should be kept in a tightly closed container and protected from light.

Additional information. Neomycin sulfate is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. An aqueous solution is dextrorotatory.

Requirements

Definition. Neomycin sulfate is a mixture of sulfate salts of substances produced by the growth of *Streptomyces fradiae*, the main components of which are neomycin B and its stereoisomer neomycin C.

Neomycin sulfate contains not less than 600 International Units of neomycin per mg, calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and freshly prepared ammonium acetate (40 g/l) TS as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions containing (A) 20 mg of the test substance per ml, and (B) 20 mg of neomycin B sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes, heat at 105 °C for 1 hour, and spray with triketohydrindene/butanol TS. Heat it again at 105 °C for 5 minutes and examine the chromatogram in daylight. The principal red spot obtained with solution A corresponds in position and appearance with that obtained with solution B.

B. Dissolve 10 mg in 5 ml of water, add 0.1 ml of pyridine R and 2 ml of triketohydrindene hydrate (1 g/l) TS, and heat on a water-bath at a temperature between 65 and 70 °C for 10 minutes; a deep violet colour is produced.

C. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Sulfated ash. Not more than 10 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 80 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 5.0–7.5.

Neamine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate prepared as follows: Mix 0.3 g of carbomer R with 240 ml of water and allow to stand for 1 hour with occasional moderate shaking. Adjust the pH to 7 by slowly adding, with continuous shaking, sodium hydroxide (~80 g/l) TS, then add 30 g of silica gel R3. Coat the plate with a layer of 0.75 mm thickness, heat the plate at 110 °C for 1 hour, allow to cool, and use immediately. As the mobile phase, use potassium dihydrogen phosphate (100 g/l) TS. Apply separately to the plate 10 µl of each of 2 solutions containing (A) 2.5 mg of the test substance per ml and (B) 0.05 mg of neamine hydrochloric RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, spray it with triketohydrindene/stannous chloride TS, and heat it at 110 °C for 15 minutes. Examine the chromatogram in daylight. Any spot obtained with solution A corresponding to neamine is not more intense than that obtained with solution B.

Content of sulfates. Dissolve 1 g in 200 ml of water, add 3 ml of hydrochloric acid (~420 g/l) TS, heat to boiling, and add 25 ml of hot barium chloride (50 g/l) TS. Heat on a water-bath for 4 hours with stirring, collect the precipitate, wash with water, dry, ignite, and weigh. Each g of residue is equivalent to 411.6 mg of sulfates; the sulfate content is not less than 250 mg/g and not more than 310 mg/g.

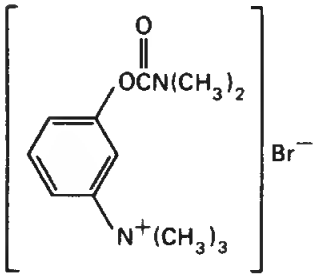
Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241; ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer pH 8.0 TS1 or TS2, an appropriate concentration of neomycin (usually between 2 and 14 IU per ml), and an incubation temperature of 35–39 °C; or (*b*) *Staphylococcus aureus* (ATCC 29737) as the test organism, culture medium Cm1 with a final pH of 7.8–8.0, sterile phosphate buffer pH 8.0 TS1 or TS2, an appropriate concentration of neomycin (usually between 2 and 20 IU per ml), and an incubation temperature of 35–39 °C; or (*c*) *Staphylococcus epidermidis* (ATCC 12228) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer pH 8.0 TS1 or TS2, an appropriate concentration of neomycin (usually between 0.5 and 2 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 600 IU of neomycin per mg, calculated with reference to the dried substance.

NEOSTIGMINI BROMIDUM
NEOSTIGMINE BROMIDE

Molecular formula. $C_{12}H_{19}BrN_2O_2$

Relative molecular mass. 303.2

Graphic formula.



Chemical name. (*m*-Hydroxyphenyl)trimethylammonium bromide dimethylcarbamate; 3-[[(dimethylamino)carbonyl]oxy]-*N,N,N*-trimethylbenzenaminium bromide; CAS Reg. No. 114-80-7.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cholinergic.

Storage. Neostigmine bromide should be kept in a tightly closed container, protected from light.

Requirements

Definition. Neostigmine bromide contains not less than 98.0% and not more than 101.0% of $C_{12}H_{19}BrN_2O_2$, calculated with reference to the dried substance.

Identity tests

A. Heat 0.05 g with 0.4 g of potassium hydroxide R and 2 ml of ethanol (~750 g/l) TS on a water-bath for 3 minutes. Replace the evaporated ethanol, cool, and add 2 ml of water and 2 ml of diazobenzenedisulfonic acid TS; a red colour is produced.

B. To a solution of 0.1 g in 5 ml of water add 15 ml of trinitrophenol (7 g/l) TS, wash the precipitate with water, and dry at 105 °C; melting temperature, about 185 °C (picrate).

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of bromides.

Sulfates. Dissolve 2.5 g in 40 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Sulfated ash. Not more than 1.5 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Acidity. Dissolve 0.2 g in 20 ml of carbon-dioxide-free water R and titrate to pH 7.0 with sodium hydroxide (0.02 mol/l) VS; not more than 0.1 ml is required.

3-Hydroxyphenyltrimethylammonium bromide. The absorbance of a 1-cm layer of a freshly prepared 5.0 mg/ml solution in sodium carbonate (10 g/l) TS at 294 nm is not more than 0.2 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

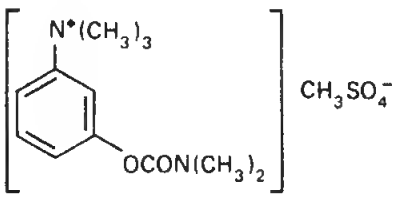
Assay. Dissolve about 0.25 g, accurately weighed, in 20 ml of glacial acetic acid R1, add 5 ml of acetic anhydride R and 10 ml of mercuric acetate/acetic acid TS. Titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

NEOSTIGMINI METILSULFAS
NEOSTIGMINE METILSULFATE

Molecular formula. $C_{13}H_{22}N_2O_6S$

Relative molecular mass. 334.4

Graphic formula.



Chemical name. (*m*-Hydroxyphenyl)trimethylammonium methyl sulfate dimethylcarbamate; 3-[[(dimethylamino)carbonyl]oxy]-*N,N,N*-trimethylbenzenaminium methyl sulfate; CAS Reg. No. 51-60-5.

Description. A white, crystalline powder; odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS.

Category. Cholinergic.

Storage. Neostigmine metilsulfate should be kept in a tightly closed container, protected from light.

Requirements

Definition. Neostigmine metilsulfate contains not less than 98.0% and not more than 100.5% of $C_{13}H_{22}N_2O_6S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from neostigmine metilsulfate RS or with the *reference spectrum* of neostigmine metilsulfate.

B. See the test described under "Related substances". The spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Heat 0.05 g with 0.4 g of potassium hydroxide R and 2 ml of ethanol (~750 g/l) TS on a water-bath for 3 minutes. Adjust to the original volume with ethanol (~750 g/l) TS, cool, and add 2 ml of water and 2 ml of diazobenzenedisulfonic acid TS; a red colour is produced.

D. Mix 20 mg with 0.5 g of sodium carbonate R and heat to fusion in a small crucible; boil the fused mass with 10 ml of water until it has disintegrated, then filter. Add 0.2 ml of bromine TS1 to the filtrate, heat to boiling, acidify with hydrochloric acid (~70 g/l) TS, and expel the excess of bromine by boiling; the resulting solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Melting range. 144–149 °C, after drying at 105 °C for 3 hours.

Chlorides. Dissolve 0.20 g in 10 ml of water, add 1 ml of nitric acid (~130 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; no immediate appearance of opalescence is observed.

Sulfates. Dissolve 0.20 g in 10 ml of water, add 1.5 ml of hydrochloric acid (~70 g/l) TS and 1 ml of barium chloride (50 g/l) TS; no ready appearance of turbidity is produced.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and not more intensely coloured than standard colour solution BnI when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Acidity. Dissolve 0.20 g in 10 ml of water and add 0.1 ml of phenolphthalein/ethanol TS; the test solution is colourless. Add 0.30 ml of sodium hydroxide (0.01 mol/l) VS; the solution turns red.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 67 volumes of water, 30 volumes of methanol R, and 3 volumes of diethylamine R as the mobile phase (a certain type of precoated plate may not be suitable with this mobile phase). Apply separately to the plate 10 µl of each of 3 solutions containing (A) 20 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of neostigmine metilsulfate RS per ml. After removing the plate from the chromatographic chamber allow it to dry in a current of warm air, spray it with 4-nitroaniline TS2 and then with sodium hydroxide (0.1 mol/l) VS. Dry the plate again in a current of warm air, spray it with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.15 g, accurately weighed, in 20 ml of water, transfer to an ammonia semi-micro distillation apparatus, and add 25 ml of sodium hydroxide (~400 g/l) TS. Pass a current of steam through the mixture and collect the distillate in 50 ml of sulfuric acid (0.01 mol/l) VS until the total volume reaches about 200 ml. Titrate the excess of acid with sodium hydroxide (0.02 mol/l) VS using methyl red/ethanol TS as indicator. Repeat the operation without the substance being tested; the difference between the titrations represents the amount of acid required to neutralize the dimethylamine formed from the neostigmine. Each ml of sulfuric acid (0.01 mol/l) VS is equivalent to 6.688 mg of $C_{13}H_{22}N_2O_6S$.

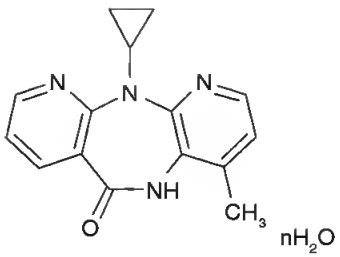
Additional requirement for Neostigmine metilsulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

NEVIRAPINUM

NEVIRAPINE

Nevirapine, anhydrous
Nevirapine hemihydrate



n = 0 (anhydrous)
n = ½ (hemihydrate)

Molecular formula. $C_{15}H_{14}N_4O$ (anhydrous); $C_{15}H_{14}N_4O,\frac{1}{2}H_2O$ (hemihydrate)

Relative molecular mass. 266.3 (anhydrous); 275.3 (hemihydrate)

Chemical name. 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one; CAS Reg. No. 129618-40-2.(anhydrous) 11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one hemihydrate

Description. A white to almost white powder.

Solubility. Practically insoluble in water, sparingly to slightly soluble in dichloromethane R, slightly soluble in methanol R.

Category. Antiretroviral (Non-Nucleoside Reverse Transcriptase Inhibitor).

Storage. Nevirapine should be kept in a well closed container.

Labelling. The designation on the container should state whether the substance is the hemihydrate or is in the anhydrous form.

Requirements

Nevirapine contains not less than **98.0%** and not more than **102.0%** of $C_{15}H_{14}N_4O$, calculated with reference to the anhydrous substance.

Identity test

- Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of anhydrous nevirapine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of anhydrous nevirapine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in basic potassium permanganate (~1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 220 nm and 350 nm, exhibits a maximum at about 283 nm with a specific absorbance ($A^{1\%}_{1\,cm}$), calculated with reference to the anhydrous substance, between 251 and 285.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For the anhydrous substance, the infrared absorption spectrum is concordant with the spectrum obtained from anhydrous nevirapine RS or with the *reference spectrum* of anhydrous nevirapine For the hemihy-

drate, the infrared absorption spectrum shows a characteristic sharp absorbance at about 3503 cm^{-1}; after heating the test substance for one hour at 140 °C and cooling, the infrared absorption spectrum is concordant with the spectrum obtained from anhydrous nevirapine RS or with the *reference spectrum* of anhydrous nevirapine.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. For the anhydrous form, use about 1 g of the test substance; the water content is not more than 2 mg/g. For the hemihydrate, use about 0.30 g of the test substance; the water content is not less than 31 mg/g and not more than 39 mg/g.

Related substances
Prepare fresh solutions and perform the tests without delay

Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (15 cm × 4.6 mm), packed with hexadecylamidylsilyl silica gel for chromatography (5 µm).

Maintain the column temperature at 35 °C.

The mobile phase consists of a filtered and degassed mixture of 20 volumes of acetonitrile R and 80 volumes of a 3.6 g/l solution of ammonium dihydrogen phosphate R adjusted to pH 5.0 using ammonia (~260 g/l) TS.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 220 nm.

Prepare the following solutions. For solution (1) dissolve 24 mg of the test substance in 4 ml of acetonitrile R, add 80 ml of the mobile phase and sonicate. Dilute to 100.0 ml with the mobile phase. For solution (2) dilute 5.0 ml of solution (1) to 50.0 ml with the mobile phase. Then dilute 5.0 ml of this solution to 50.0 ml with the same solvent. For solution (3) dissolve 6 mg of nevirapine impurity B RS in a mixture of 25 ml of acetonitrile R and 55 ml of mobile phase, sonicate for 30 min and dilute to 100.0 ml with the mobile phase. Then mix 6.0 ml of this solution with 3.0 ml of solution (1) and dilute to 50.0 ml with the mobile phase. For solution (4) dissolve 12 mg of anhydrous nevirapine RS in 2 ml of acetonitrile R and 40 ml of the mobile phase and sonicate. Dilute to 50.0 ml with the mobile phase. Then dilute 3.0 ml of this solution to 25.0 ml with the mobile phase. For solution (5) Dilute 3.0 ml of solution (1) to 25.0 ml with the mobile phase.

Inject separately 50 µl of solution (2) in replicate injections in the chromatographic system. The relative standard deviation for peak areas of nevirapine in

replicate injections of solution (2) is not more than 5.0%. The test is not valid unless the column efficiency determined for nevirapine using solution (2) is not less than 10 000. The peak symmetry factor of nevirapine should be between 0.8 and 1.2.

Inject 50 µl of solution (3). The test is not valid unless the resolution between nevirapine and nevirapine impurity B RS is not less than 5.

Inject separately 50 µl each of solution (1) and of mobile phase in the chromatographic system and record the chromatograms for 6 times the retention time of nevirapine. Examine the mobile phase chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (1).

In the chromatogram obtained with solution (1), the following impurity peaks are eluted at the following relative retention with reference to nevirapine (retention time about 7.6 min): impurity B about 0.7; impurity A about 1.5; impurity C about 2.8.

In the chromatogram obtained with solution (1) the area of any individual peak corresponding to impurity A or C is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the area of any individual peak corresponding to impurity B, when multiplied by a correction factor of 0.77, is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%).The area of any other impurity peak is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with solution (2) (0.6%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay

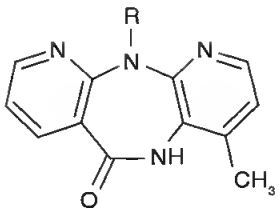
Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given under Related substances.

Inject alternatively 25 µl of solutions (4) and (5).

Measure the areas of the peaks responses obtained in the chromatogram from solutions (4) and (5), and calculate the percentage content of $C_{15}H_{14}N_4O$ with reference to the anhydrous substance.

Impurities

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.



A. R = C_2H_5: 11-ethyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4] diazepin-6-one,

B. R = H: 4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one,

C. R = CH_2-CH_2-CH_3: 4-methyl-11-propyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′, 3′-*e*][1,4]diazepin-6-one.

NICLOSAMIDUM

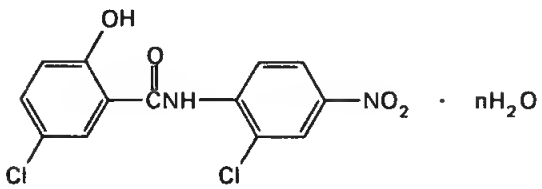
NICLOSAMIDE

Niclosamide, anhydrous
Niclosamide monohydrate

Molecular formula. $C_{13}H_8Cl_2N_2O_4$ (anhydrous); $C_{13}H_8Cl_2N_2O_4,H_2O$ (monohydrate)

Relative molecular mass. 327.1 (anhydrous); 345.1 (monohydrate)

Graphic formula.



n = 0 (anhydrous)
n = 1 (monohydrate)

Chemical name. 2′,5-Dichloro-4′-nitrosalicylanilide; 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide; CAS Reg. No. 50-65-7 (anhydrous).

2′,5-Dichloro-4′-nitrosalicylanilide monohydrate; 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide monohydrate; CAS Reg. No. 73360-56-2 (monohydrate).

Description. A cream-coloured, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 150 parts of ethanol (~750 g/l) TS; slightly soluble in ether R, and acetone R.

Category. Taeniacide.

Storage. Niclosamide should be kept in a tightly closed container.

Labelling. The designation on the container of Niclosamide should state whether the substance is the monohydrate or is in the anhydrous form.

Additional information. Anhydrous Niclosamide is hygroscopic.

Requirements

Definition. Niclosamide contains not less than 98.0% and not more than 100.5% of $C_{13}H_8Cl_2N_2O_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of a relevant form of niclosamide.

B. Dissolve 1 mg in 2 ml of dimethylformamide R and add 2 drops of potassium hydroxide/ethanol TS1; a strong red colour is produced.

C. Dissolve 0.1 g in 1 ml of acetic anhydride R and boil for 10 minutes. Cool and add 10 ml of water. Collect the precipitate on a filter, wash with water, recrystallize from ethanol (~750 g/l) TS, and dry at 105 °C; melting temperature, about 178 °C (acetyl-derivative).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C. Anhydrous Niclosamide loses not more than 5.0 mg/g. Niclosamide monohydrate loses not less than 40 mg/g and not more than 60 mg/g.

Acidity or alkalinity. Boil 0.8 g in 40 ml of water for 1 minute and filter. To 10 ml of the filtrate add 2 drops of phenolphthalein/ethanol TS and 0.2 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS; a red colour is produced. Add 5 drops of methyl red/ethanol TS and 0.4 ml of hydrochloric acid (0.01 mol/l) VS; the colour of the solution changes from red to orange.

2-Chloro-4-nitroaniline. Boil 0.1 g with 20 ml of methanol R for 2 minutes, cool, add sufficient hydrochloric acid (1 mol/l) VS to produce 50 ml, and filter. To 10 ml of the filtrate add 1.0 ml of sodium nitrite (3 g/l) TS and allow to stand for 10 minutes; add 1 ml of ammonium sulfamate (25 g/l) TS, shake, allow to stand for 10 minutes, and add 1 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS. Treat similarly 10 µg of 2-chloro-4-nitroaniline R. The colour produced in the test solution is not more intense than that of the reference solution when compared as described under 1.11 Colour of liquids.

5-Chlorosalicylic acid. Boil 0.5 g with 10 ml of water for 2 minutes, cool, filter, and add to the filtrate a few drops of ferric chloride (25 g/l) TS; no red or violet colour is produced.

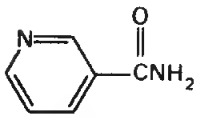
Assay. Dissolve about 0.3 g, accurately weighed, in 60 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B. Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

NICOTINAMIDUM
NICOTINAMIDE

Molecular formula. $C_6H_6N_2O$

Relative molecular mass. 122.1

Graphic formula.



Chemical name. 3-Pyridinecarboxamide; 3-pyridinecarboxylic acid amide; CAS Reg. No. 98-92-0.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Soluble in 1 part of water and 2 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Vitamin.

Storage. Nicotinamide should be kept in a well-closed container.

Requirements

Definition. Nicotinamide contains not less than 99.0% and not more than 101.0% of $C_6H_6N_2O$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nicotinamide RS or with the *reference spectrum* of nicotinamide.

B. Dissolve 10 mg in 10 ml of water. To 2 ml add 2 ml of thiocyanate reagent, obtained by adding, drop by drop, ammonium thiocyanate (0.1 mol/l) VS to bromine TS1 until the yellow coloration disappears. Then add 3 ml of aniline (25 g/l) TS and shake; a yellow colour is produced.

C. Boil gently 0.1 g with 1 ml of sodium hydroxide (~80 g/l) TS in a test-tube; ammonia, perceptible by its odour, is evolved.

Melting range. 128–131 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 30 µg/g.

Clarity and colour of solution. A solution of 2.5 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R or phosphorus pentoxide R; it loses not more than 5.0 mg/g.

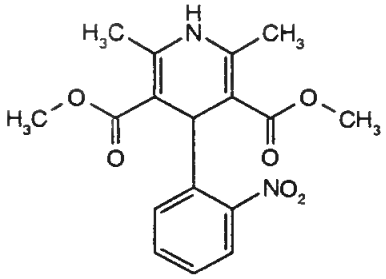
pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 6.0–8.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 48 volumes of chloroform R, 10 volumes of water and 45 volumes of dehydrated ethanol R as the mobile phase. Apply separately to the plate 5 µl of each

of 2 solutions in a mixture of equal volumes of ethanol (~750 g/l) TS and water containing (A) 0.12 g of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.25 g, accurately weighed, in 20 ml of glacial acetic acid R1, add 5 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 12.21 mg of $C_6H_6N_2O$.

NIFEDIPINUM
NIFEDIPINE



$C_{17}H_{18}N_2O_6$

Relative molecular mass. 346.3

Chemical name. 1,4-Dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridinedicarboxylate dimethyl ester; 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester; CAS Reg. No. 21829-25-4.

Description. A yellow, crystalline powder.

Solubility. Nifedipine is practically insoluble in water; freely soluble in acetone R; sparingly soluble in dehydrated ethanol R.

Category. Cardiovascular drug; calcium-channel blocking agent.

Storage. Nifedipine should be kept in a tightly closed container, protected from light.

Additional information. *CAUTION*: Nifedipine decomposes on exposure to daylight, artificial light of certain wavelengths, and ultraviolet light.

Requirements

Nifedipine contains not less than **98.0%** and not more than **102.0%** of $C_{17}H_{18}N_2O_6$, calculated with reference to the dried substance.

Note: Throughout the monograph perform the tests and the assay in the dark or under a suitable fluorescent light, using low-actinic glassware.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nifedipine RS or with the *reference spectrum* of nifedipine.

B. See the test described below under "Related substances, Test B". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Using gentle heat dissolve 25 mg in 10 ml of a mixture of 5 volumes of ethanol (~750 g/l) TS, 3.5 volumes of water, and 1.5 volumes of hydrochloric acid (~420 g/l) TS. Add 0.5 g of granulated zinc R and allow to stand for 5 minutes, swirling occasionally, and filter. To the filtrate add 5 ml of sodium nitrite (10 g/l) TS and allow to stand for 2 minutes. Add 2.0 ml of ammonium sulfamate (50 g/l) TS, shake vigorously but carefully, and add 2.0 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS; an intense red colour is produced which does not fade within 5 minutes.

D. Melting temperature, about 173 °C.

Sulfated ash. Use an ignition temperature of 600 °C; not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 5.0 mg/g.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (15 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5–10 µm). As the mobile phase, use a mixture of 55 volumes of water, 36 volumes of methanol R, and 9 volumes of acetonitrile R.

Prepare the following solutions in methanol R: for solution (A) dissolve 0.20 g of Nifedipine in 20 ml of methanol R and dilute to 50 ml with the mobile phase; solution (B) 0.4 mg of dimethyl 2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate RS per ml; solution (C) 0.4 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate RS 120 per ml; and for solution (D) mix 1.0 ml each of solutions B and C and 0.10 ml of solution A, dilute to 20 ml with the mobile phase, then dilute 2.0 ml of the resulting solution to 10 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 235 nm; the use of an electronic integrator is advisable.

Inject 20 µl of solution D. The peaks are eluted in the following order: dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate; dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate; and nifedipine. The retention time of nifedipine is about 15.5 minutes.

The test is not valid unless, in the chromatogram obtained with solution D:

— the resolution between the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is greater than 1.5; and

— the resolution between the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is greater than 1.5.

Adjust the sensitivity of the system so that the height of the peak corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate is not less than 20% of the full scale of the recorder.

Inject alternately 20 µl each of solutions A and D. Record the chromatogram for twice the retention time of nifedipine.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and D, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, none of the peaks, other than the principal peak and the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate, has an area greater than that of the peak corresponding to nifedipine in the chromatogram obtained with solution D (0.1%). The areas of the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-

dicarboxylate are not greater than the corresponding peaks in the chromatogram obtained with solution D (0.1%). The total amount of related substances does not exceed 0.3%. Disregard any peak with an area less than 10% of the area of the peak corresponding to nifedipine in the chromatogram obtained with solution D (0.01%).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, in an unsaturated chamber, using silica gel R6 as the coating substance and a mixture of 6 volumes of cyclohexane R and 4 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 1.0 µg of Nifedipine per ml, (B) 1.0 mg of nifedipine RS per ml, and (C) 10 µg of Nifedipine per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (1.0%).

Assay. Dissolve about 0.13 g, accurately weighed, in a mixture of 25 ml of *tert*-butanol R and 25 ml of perchloric acid TS, and titrate with ceric ammonium sulfate (0.1 mol/l) VS, using 0.1 ml of ferroin TS as indicator until the pink colour is discharged, titrating slowly towards the end-point. Repeat the procedure without the Nifedipine being examined and make any necessary corrections.

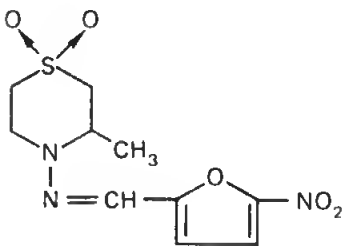
Each ml of ceric ammonium sulfate (0.1 mol/l) VS is equivalent to 17.32 mg of $C_{17}H_{18}N_2O_6$.

NIFURTIMOXUM
NIFURTIMOX

Molecular formula. $C_{10}H_{13}N_3O_5S$

Relative molecular mass. 287.3

Graphic formula.



Chemical name. 3-Methyl-4-[(5-nitrofurfurylidene)amino]thiomorpholine 1,1-dioxide; 3-methyl-*N*-[(5-nitro-2-furanyl)methylene]-4-thiomorpholinamine 1,1-dioxide; CAS Reg. No. 23256-30-6.

Description. A yellow to orange-yellow, crystalline powder; odourless.

Solubility. Practically insoluble in water and ether R; freely soluble in dimethylformamide R; sparingly soluble in dioxan R.

Category. Antitrypanosomal drug.

Storage. Nifurtimox should be kept in a well-closed container.

Requirements

Definition. Nifurtimox contains not less than 98.0% and not more than 102.0% of $C_{10}H_{13}N_3O_5S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nifurtimox RS or with the *reference spectrum* of nifurtimox.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Dissolve 20 mg in a mixture of 2.0 ml of dimethylformamide R and 2.0 ml of water. Add 0.25 ml of copper(II) sulfate (160 g/l) TS and 4 drops of pyridine R; a dark green colour is produced. Add 5 ml of chloroform R and shake; the green colour remains in the aqueous layer.

Melting range. 178–182 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of equal volumes of ethyl acetate R and hexane R as the mobile phase. Apply separately to the plate the following 3 solutions in acetone R: (A) 10 µl containing 10 mg of the test sub-

stance per ml, (B) 2 µl containing 1.0 mg of the test substance per ml, and (C) 2 µl containing 1.0 mg of nifurtimox RS per ml. Allow the spots to dry before placing the plate into an unsaturated chromatographic chamber and develop the plate for a distance of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

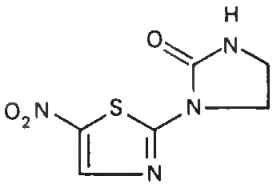
Assay. Dissolve about 0.1 g, accurately weighed, in 20 ml of dimethylformamide R and 80 ml of ethanol (~750 g/l) TS. Add 5 ml of hydrochloric acid (~250 g/l) TS and start to bubble carbon dioxide R into the solution, keeping it up throughout the determination. After 3 minutes add 30.0 ml of titanium trichloride (0.1 mol/l) VS and 3 ml of potassium thiocyanate (200 g/l) TS. Allow to stand for 15 minutes and titrate the excess titanium trichloride with ferric ammonium sulfate (0.1 mol/l) VS. Each ml of titanium trichloride (0.1 mol/l) VS is equivalent to 4.788 mg of $C_{10}H_{13}N_3O_5S$.

NIRIDAZOLUM
NIRIDAZOLE

Molecular formula. $C_6H_6N_4O_3S$

Relative molecular mass. 214.2

Graphic formula.



Chemical name. 1-(5-Nitro-2-thiazolyl)-2-imidazolidinone; CAS Reg. No. 61-57-4.

Description. A yellow, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water and ether R; soluble in dimethylformamide R and pyridine R; slightly soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antischistosomal drug.

Storage. Niridazole should be kept in a tightly closed container.

Requirements

Definition. Niridazole contains not less than 97.0% and not more than 103.0% of $C_6H_6N_4O_3S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from niridazole RS or with the *reference spectrum* of niridazole.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 20 mg in 2.0 ml of acetone R, add 2.0 ml of sodium hydroxide (~80 g/l) TS, shake, and allow to stand for 10 minutes; a dark red colour is produced in the lower layer.

D. Melting temperature, about 264 °C with decomposition.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfates. Add 0.5 g of the test substance to a mixture of 2.0 ml of hydrochloric acid (~70 g/l) TS and 8.0 ml of water, heat to boiling, cool to room temperature, filter, and dilute the filtrate to 10 ml with water; the solution is clear. Add 1.0 ml of barium chloride (0.5 mol/l) VS and again heat to boiling.

Similarly prepare a comparison solution containing 0.10 ml of sulfuric acid (0.01 mol/l) VS, 2.0 ml of hydrochloric acid (~70 g/l) TS, 8.0 ml of water, 1.0 ml of barium chloride (0.5 mol/l) VS, and heat to boiling.

Allow both solutions to stand for 1 hour; the opalescence in the solution prepared from the test substance is not more intense than that produced in the comparison solution.

Solution in dimethylformamide. A solution of 0.10 g in 10 ml of dimethylformamide R is clear.

Sulfated ash. Not more than 3.0 mg/g.

Loss on drying. Dry at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 12 volumes of toluene R and 8 volumes of acetone R as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions in pyridine R containing (A) 10 mg of the test substance per ml (heat slightly to dissolve, if necessary), (B) 10 mg of niridazole RS per ml (heat slightly to dissolve, if necessary), (C) 20 µg of 2-amino-5-nitrothiazole RS per ml, and (D) 40 µg of niridazole-chlorethylcarboxamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air; examine the chromatogram immediately in ultraviolet light (365 nm) and again after 15 minutes' irradiation. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C viewed immediately and not more intense than that obtained with solution D viewed after 15 minutes.

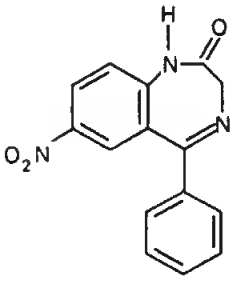
Assay. Dissolve about 40 mg, accurately weighed, in 4 ml of dimethylformamide R and dilute with sufficient dehydrated ethanol R to produce 200 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 359 nm. Calculate the amount of $C_6H_6N_4O_3S$ in the substance being tested by comparison with niridazole RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of a 10 µg/ml solution of niridazole RS should be 0.70 ± 0.03.

NITRAZEPAMUM
NITRAZEPAM

Molecular formula. $C_{15}H_{11}N_3O_3$

Relative molecular mass. 281.3

Graphic formula.



Chemical name. 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one; CAS Reg. No. 146-22-5.

Description. A yellow, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Sedative; hypnotic.

Storage. Nitrazepam should be kept in a well-closed container, protected from light.

Requirements

Definition. Nitrazepam contains not less than 98.5% and not more than 101.0% of $C_{15}H_{11}N_3O_3$, calculated with reference to the dried substance.

Identity tests

A. NOTE: Carry out the following operations in subdued light. Prepare a 0.005 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 9 volumes of methanol R and examine immediately. The absorption spectrum, when observed between 230 nm and 350 nm, exhibits a maximum only at about 280 nm; the absorbance of a 1-cm layer at this wavelength is about 0.45.

B. Dissolve 10 mg in 1 ml of methanol R, warming if necessary, and add 0.05 ml of sodium hydroxide (~80 g/l) TS; an intense yellow colour is produced.

C. Dissolve 20 mg in a mixture of 5 ml of hydrochloric acid (~420 g/l) TS and 10 ml of water, boil for 5 minutes, cool, and add 2 ml of sodium nitrite (1 g/l) TS. Allow to stand for 1 minute, add 1 ml of sulfamic acid (5 g/l) TS, mix, allow to stand for 1 minute, and add 1 ml of N-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS; a red colour is produced.

D. Melting temperature, about 227 °C with decomposition.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test in subdued light as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 85 volumes of nitromethane R and 15 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of 2 freshly prepared solutions in a mixture of equal volumes of chloroform R and methanol R containing (A) 25 mg of the test substance per ml and (B) 25 µg of the test substance per ml. Develop the plate for a distance of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.25 g, accurately weighed, in 30 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 28.13 mg of $C_{15}H_{11}N_3O_3$.

NITROFURANTOINUM

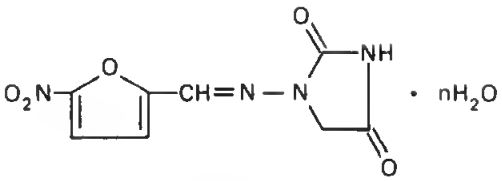
NITROFURANTOIN

Nitrofurantoin, anhydrous
Nitrofurantoin monohydrate

Molecular formula. $C_8H_6N_4O_5$ (anhydrous); $C_8H_6N_4O_5,H_2O$ (monohydrate).

Relative molecular mass. 238.2 (anhydrous); 256.2 (monohydrate).

Graphic formula.



n = 0 (anhydrous)
n = 1 (monohydrate)

Chemical name. 1-[(5-Nitrofurfurylidene)amino]hydantoin; 1-[[(5-nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione; CAS Reg. No. 67-20-9 (anhydrous).

1-[(5-Nitrofurfurylidene)amino]hydantoin monohydrate; 1-[[(5-nitro-2-furanyl)-methylene]amino]-2,4-imidazolidinedione monohydrate; CAS Reg. No. 17140-81-7 (monohydrate).

Other name. Furadoninum.

Description. Lemon-yellow crystals or a yellow, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; very slightly soluble in ethanol (~750 g/l) TS; soluble in dimethylformamide R.

Category. Antibacterial drug.

Storage. Nitrofurantoin should be kept in a well-closed container, protected from light, and stored at a temperature not exceeding 25 °C.

Labelling. The designation on the container of Nitrofurantoin should state whether the substance is the monohydrate or is in the anhydrous form.

Additional information. Nitrofurantoin melts at about 271 °C with decomposition. Nitrofurantoin and its solutions are discoloured by alkali and by exposure to light and are decomposed upon contact with metals other than stainless steel and aluminium.

Requirements

Definition. Nitrofurantoin contains not less than 98.0% and not more than 102.0% of $C_8H_6N_4O_5$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. For Nitrofurantoin monohydrate, the substance must be previously dried to constant weight at 105 °C. The infrared absorption spectrum is concordant with the spectrum obtained from nitrofurantoin RS or with the *reference spectrum* of nitrofurantoin.

B. Carry out the following operations in subdued light.

Prepare a blank solution by dissolving 3.6 g of sodium acetate R in 20 ml of water, adding 0.3 ml of glacial acetic acid R, and diluting with sufficient water to produce 200 ml. Dissolve 0.12 g in 50 ml of dimethylformamide R and add sufficient water to produce 1000 ml. Dilute 5 ml of this solution to 100 ml with the blank solution prepared above. Measure the absorption spectrum of the resulting solution against 10 ml of the blank solution to which 0.05 ml of dimethylformamide R has been added. When observed between 220 nm and 400 nm, the absorption spectrum exhibits maxima at about 266 nm and 367 nm. The absorbances of a 1-cm layer at these wavelengths are about 0.32 and 0.46, respectively. The ratio of the absorbance at 367 nm to that at 266 nm is between 1.36 and 1.42.

C. Dissolve 0.2 g in a mixture of 10 ml of dimethylformamide R and 1 drop of glacial acetic acid R. To 0.5 ml of this solution add 2 ml of water, 0.15 ml of copper(II) sulfate (80 g/l) TS, and 0.2 ml of pyridine R; shake with 3 ml of chloroform R and allow the layers to separate; a green colour is produced in the chloroform layer.

D. Dissolve 5 mg in 5 ml of sodium hydroxide (0.1 mol/l) VS; a deep yellow solution is produced, which becomes deep orange-red on standing.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; anhydrous Nitrofurantoin loses not more than 10 mg/g. Nitrofurantoin monohydrate loses not less than 50 mg/g and not more than 71 mg/g.

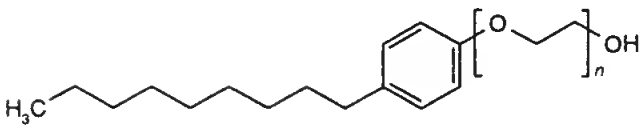
Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 90 volumes of nitromethane R and 10 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions: (A) dissolve 0.25 g of the test substance in a minimum volume of dimethylformamide R and dilute to 10 ml with acetone R; (B) dilute 1 ml of solution A to 100 ml with acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air and heat it at 105 °C for 5 minutes. Spray the warm plate with phenylhydrazine/hydrochloric acid TS, heat it again at 105 °C for 10 minutes, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained

with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4g, accurately weighed, in a mixture of 10ml of dimethylformamide R and 10ml of dioxan R, add 0.10ml of thymol blue/dimethylformamide TS and titrate with lithium methoxide (0.1mol/l) VS to a dark green endpoint, as described under 2.6 Non-aqueous titration, Method B. Each ml of lithium methoxide (0.1mol/l) VS is equivalent to 23.82mg of $C_8H_6N_4O_5$.

NONOXINOLUM 9

NONOXINOL 9



$C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$
(*Average value of n = 9, with a possible range of 4–16.*)

Chemical name. Polyethylene glycol mono(*p*-nonylphenyl) ether; α-(4-nonylphenyl)-ω-hydroxypoly-(oxy-1,2-ethanediyl); CAS Reg. No. 26027-38-3.

Description. A clear, colourless to light yellow, viscous liquid.

Solubility. Miscible with water, ethanol (~750g/l) TS and olive oil R.

Category. Adjunctive contraceptive agent.

Storage. Nonoxinol 9 should be kept in a tightly closed container.

Additional information. Nonoxinol 9 should be kept away from oxidizing agents.

Requirements

Definition. Nonoxinol 9 is an anhydrous liquid mixture containing mainly monononylphenyl ethers of macrogols.

The content is not less than **95.0%** and not more than **105.0%** of Nonoxinol 9, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of nonoxinol 9.

B. See the test described below under "Assay". The retention time of the major peak in the chromatogram obtained with solution A corresponds to that in the chromatogram obtained with solution B.

Acid value. Not more than 0.2.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

Macrogol. Transfer about 10 g, accurately weighed, to a 250-ml beaker. Add 100 ml of ethyl acetate R and allow to dissolve using a magnetic stirrer. Transfer to a 500-ml separatory funnel fitted with a glass stopper with the aid of 100 ml of sodium chloride (300 g/l) TS. Insert the stopper and shake vigorously for 1 minute. Remove the stopper carefully. Immerse a thermometer into the mixture and place the funnel so that it is partially immersed in a water-bath maintained at 50 °C. Swirl the funnel gently while letting the internal temperature rise to between 40 and 45 °C. Once this is reached remove the funnel from the bath immediately, dry the outside surface, and drain the aqueous layer into another 500-ml separatory funnel. Extract the organic layer with 100 ml of sodium chloride (300 g/l) TS a second time, combining the two aqueous extracts. Discard the organic layer. Wash the combined aqueous layers with 100 ml of ethyl acetate R and separate the aqueous layer into another 500-ml separatory funnel. Discard the organic layer. Extract the aqueous layer with two successive 100-ml portions of dichloromethane R, filtering the organic layers through a folded filter paper (e.g. grade Whatman 2V) and combining them in a 250-ml beaker. Evaporate to dryness on a water-bath and continue heating until the odour of dichloromethane is no longer perceptible. Allow the beaker to cool. Add 25 ml of acetone R and allow the residue to dissolve using a magnetic stirrer. Filter into a tared 250-ml beaker, rinsing with two 25-ml quantities of acetone R. Evaporate to dryness on a water-bath. Dry under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) at 60 °C for 1 hour. Allow the beaker to cool, and weigh; not more than 16 mg/g.

Cloudiness of solution. Transfer 1.0 g to a 250-ml beaker, add 99 g of water, and mix to dissolve. Pour about 30 ml of the solution into a 70-ml test-tube. Place the test-tube into a water-bath and stir the contents constantly with a thermometer until the solution becomes cloudy, then immediately remove the test-tube from the bath, so that the temperature does not rise further by more than 2 °C, and continue stirring. The temperature at which the solution

becomes sufficiently clear and when the entire thermometer bulb is clearly visible is between 52 and 56 °C.

Ethylene oxide and dioxan. Carry out the test as described under 1.14.5 Gas chromatography, with the apparatus equipped with an injection system for the performance of head-space chromatography. Use a capillary glass or quartz column (30 m × 0.32 mm), the inner surface of which is coated with a thick layer of polydimethylsiloxane R (1.0 µm). Maintain the temperature of the column at 50 °C for 5 minutes. Increase the temperature at a rate of 5 °C per minute to 180 °C, and then increase the temperature again at a rate of 30 °C per minute to 230 °C, and maintain it at this point for 5 minutes. Maintain the temperature of the injection port at 150 °C and that of the detector at 250 °C. Use helium R or nitrogen R as the carrier gas with a linear velocity of about 20 cm per second and a split ratio of 1 : 20; use a flame-ionization detector.

Prepare the following solutions: for solution (A) weigh 1.0 g of Nonoxinol 9, add 1.0 ml of water, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes; for solution (B) weigh 1.0 g of Nonoxinol 9, add 0.5 ml of ethylene oxide TS and 0.5 ml of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes; for solution (C) add to 0.5 ml of ethylene oxide TS 0.10 ml of a freshly prepared 10 mg/l solution of acetaldehyde R and 0.10 ml of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes.

Inject 1.0 ml of the gaseous phase of solution C. Adjust the sensitivity of the system so that the heights of the peaks corresponding to ethylene oxide and acetaldehyde in the chromatogram obtained are at least 15% of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to acetaldehyde and ethylene oxide is at least 2.0 and the peak of ethylene oxide is detected with a signal-to-noise ratio of at least 5.

Inject separately 1.0 ml each of the gaseous phases of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The mean areas of the ethylene oxide and dioxan peaks obtained with solution A are not greater than half the mean area of the corresponding peak obtained with solution B (1 mg/g of ethylene oxide and 50 mg/g of dioxan).

Assay. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of porous silica, the surface of which has been modified with chemically bonded dihydroxypropane groups (diol) (10 µm). As mobile phase A, use a mixture of 2 volumes of ethyl acetate R and 8 volumes of hexane R. As mobile phase B, use a mixture of 2.5 volumes of methanol R and 97.5 volumes of ethyl acetate R.

Prepare the following solutions in mobile phase A: solution (A) 2.0 mg of Nonoxinol 9 per ml; and solution (B) 2.0 mg of nonoxinol 9 RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 280 nm.

Use the following gradient elution system:

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0–2	100	0	equilibration
2–10	100 → 84	0 → 16	linear gradient
10–20	84 → 70	16 → 30	linear gradient
20–30	70 → 62	30 → 38	linear gradient
30–40	62 → 57	38 → 43	linear gradient
40–50	57 → 54	43 → 46	linear gradient
50–70	54 → 50	46 → 50	linear gradient
70–75	50 → 50	50 → 50	isocratic
75–76	50 → 100	50 → 0	re-equilibration

Inject 100 µl each of solutions A and B. The nonoxinol oligomers elute as sharp distinct peaks, and their areas should be included in the peak response for nonoxinol 9.

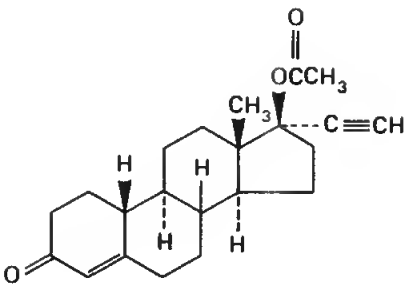
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The sum of the areas of any peaks corresponding to nonoxynols with chain lengths $n < 4$ or $n > 16$ is not greater than 1.0% of the sum of the areas of the peaks corresponding to nonoxynols with chain lengths $n = 4$ to $n = 16$. Calculate the content of Nonoxinol 9 as a percentage, with reference to the anhydrous substance.

NORETHISTERONI ACETAS
NORETHISTERONE ACETATE

Molecular formula. $C_{22}H_{28}O_3$

Relative molecular mass. 340.5

Graphic formula.



Chemical name. 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one acetate; 17-(acetyloxy)-19-nor-17α-pregn-4-en-20-yn-3-one; 17α-ethynyl-17-hydroxyestr-4-en-3-one acetate; CAS Reg. No. 51-98-9.

Description. A white or creamy white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 12.5 parts of ethanol (~750 g/l) TS and in 4 parts of acetone R; sparingly soluble in ether R.

Category. Progestational steroid.

Storage. Norethisterone acetate should be kept in a well-closed container, protected from light.

Requirements

Definition. Norethisterone acetate contains not less than 97.0% and not more than 103.0% of $C_{22}H_{28}O_3$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from norethisterone acetate RS or with the *reference spectrum* of norethisterone acetate.

B. See the test described under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. Heat 0.1 g with 2 ml of potassium hydroxide/ethanol (0.5 mol/l) VS in a water-bath for 5 minutes. Cool, add 2 ml of sulfuric acid (~700 g/l) TS and boil gently for 1 minute; ethyl acetate, perceptible by its odour (proceed with caution) is produced.

Specific optical rotation. Use a 20 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = -32$ to $-38°$

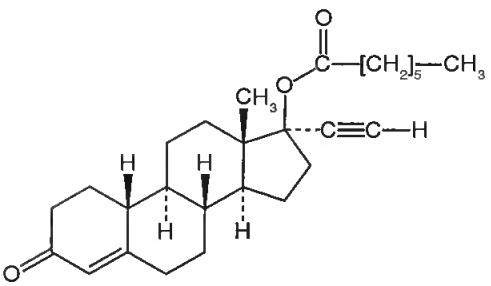
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of toluene R and ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl, in two portions of 5 µl, of each of 3 solutions in chloroform R containing (A) 10 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of norethisterone acetate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and spray with sulfuric acid/ethanol TS. Heat the plate to 105 °C for 15 minutes, allow to cool, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 10 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 10.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 240 nm. Calculate the amount of $C_{22}H_{28}O_3$ in the substance being tested by comparison with norethisterone acetate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.51 ± 0.03.

NORETHISTERONI ENANTAS
NORETHISTERONE ENANTATE



$C_{27}H_{38}O_3$

Relative molecular mass. 410.6

Chemical names. 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one heptanoate; 17-[(1-oxoheptyl)oxy]-19-nor-17α-pregn-4-en-20-yn-3-one; CAS Reg. No. 3836-23-5.

Other name. Norethindrone enantate.

Description. A white to creamy white, crystalline powder; odourless.

Solubility. Practically insoluble in water; freely soluble in acetone R, methanol R, dehydrated ethanol R, dioxan R, and ether R; slightly soluble in light petroleum R.

Category. Contraceptive.

Storage. Norethisterone enantate should be kept in a tightly closed container, protected from light.

Requirements

Norethisterone enantate contains not less than **96.0%** and not more than the equivalent of **104.0%** of $C_{27}H_{38}O_3$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from norethisterone enantate RS or with the *reference spectrum* of norethisterone enantate.

B. The absorption spectrum of a 13.5 µg/ml solution in methanol R, when observed between 210 nm and 290 nm, exhibits a maximum at about 240 nm.

C. Dissolve 1 mg in 1 ml of dehydrated ethanol R and add 0.5 ml of sulfuric acid (~1760 g/l) TS; a violet solution is produced with a red fluorescence.

Melting range. 68–73 °C.

Specific optical rotation. Use a 20 mg/ml solution in chloroform R; $[\alpha]_D^{20\,°C}$ = −10.0 to −15.0 °.

Solution in chloroform. A solution of 0.2 g in 10 ml of chloroform R is clear and almost colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over silica gel, desiccant, R at ambient temperature for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 2 volumes of cyclohexane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in chloroform R containing (A) 20 mg of Norethisterone enantate per ml and (B) 0.1 mg of Norethisterone enantate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Spray the plate with antimony trichloride TS, heat at 110 °C for 15 minutes, and examine the chromatogram in ultraviolet light (365 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Free enantic acid. Dissolve 0.3 g in 10 ml of neutralized ethanol (~750 g/l) TS using bromothymol blue/ethanol TS as indicator. Titrate the solution quickly with sodium hydroxide (0.01 mol/l) VS to a light blue endpoint; not more than 0.3 ml (corresponding to 1.3 mg/g of enantic acid).

Assay. Dissolve about 13.5 mg, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 10 ml of this solution to 100 ml with the same solvent.

Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 240 nm and calculate the content of $C_{27}H_{38}O_3$ using the absorptivity value of 42.8 ($A^{1\%}_{1\,cm}$ = 428).

Additional requirement for Norethisterone enantate for parenteral use

Complies with the monograph for "Parenteral preparations".

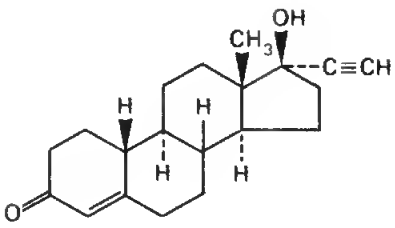
NORETHISTERONUM

NORETHISTERONE

Molecular formula. $C_{20}H_{26}O_2$

Relative molecular mass. 298.4

Graphic formula.



Chemical name. 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one; 17 α-ethynyl-17-hydroxyestr-4-en-3-one; CAS Reg. No. 68-22-4.

Description. A white or creamy white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 150 parts of ethanol (~750 g/l) TS, and in 80 parts of acetone R.

Category. Progestational steroid.

Storage. Norethisterone should be kept in a well-closed container, protected from light.

Requirements

Definition. Norethisterone contains not less than 97.0% and not more than 103.0% of $C_{20}H_{26}O_2$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from norethisterone RS or with the *reference spectrum* of norethisterone.

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

Specific optical rotation. Use a 10 mg/ml solution in chloroform R; $[\alpha]_D^{20\,°C}$ = −23 to −27 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 95 volumes of chloroform R and 5 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl in two portions of 5 µl, of each of 3 solutions in chloroform R containing (A) 10 mg of the test substance per ml, (B) 0.10 mg of the test substance per ml, and (C) 0.10 mg of norethisterone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and spray with sulfuric acid/ethanol TS. Heat the plate to 105 °C for 15 minutes, allow to cool, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

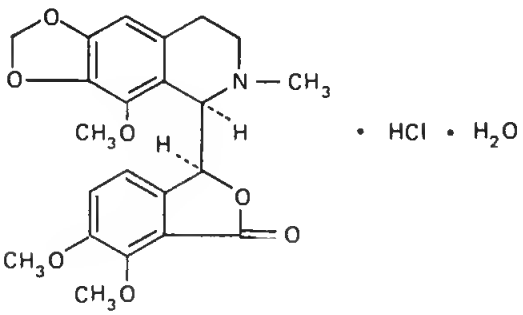
Assay. Dissolve about 10 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 10.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 240 nm. Calculate the amount of $C_{20}H_{26}O_2$ in the substance being tested by comparison with norethisterone RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.58 ± 0.03.

NOSCAPINI HYDROCHLORIDUM
NOSCAPINE HYDROCHLORIDE

Molecular formula. $C_{22}H_{23}NO_7,HCl,H_2O$

Relative molecular mass. 467.9

Graphic formula.



Chemical name. Narcotine hydrochloride monohydrate; (5*R*)-5-[(1*S*)-6,7-dimethoxyphthalidyl]-5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinoline hydrochloride monohydrate; [*S*-(*R**,*S**)]-6,7-

dimethoxy-3-(5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)-1(3*H*)-isobenzofuranone hydrochloride monohydrate; CAS Reg. No. 912-60-7 (anhydrous).

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antitussive drug.

Storage. Noscapine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Noscapine hydrochloride is hygroscopic.

Requirements

Definition. Noscapine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{22}H_{23}NO_7$,HCl, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Dissolve 0.1 g in 10 ml of water, make the solution alkaline with ammonia (~100 g/l) TS, and shake it with 10 ml of chloroform R. Separate the chloroform layer, wash it with 5 ml of water, and filter. Evaporate the filtrate almost to dryness on a water-bath, add 1 ml of dehydrated ethanol R, and evaporate to dryness. Carry out the examination using the dried residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from noscapine RS or with the *reference spectrum* of noscapine.

B. Dilute 5 ml of ammonia (~35 g/l) TS to 100 ml with methanol R; then dilute further 1 ml of this solution to 100 ml with methanol R. The absorption spectrum of a 0.050 mg/ml solution in the prepared ammonia/methanol mixture, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 291 nm and 310 nm and a minimum at about 263 nm. The ratio of the absorbance at 310 nm to that at 291 nm is between 1.2 and 1.3.

C. Place about 0.1 g on a porcelain dish, add drop by drop sulfuric acid (~1760 g/l) TS and mix; a greenish yellow colour is obtained, which turns red and finally violet on heating.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 20 mg/ml solution in hydrochloric acid (0.01 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +38.5 to +44.0 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 65 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 45 volumes of acetone R, 45 volumes of toluene R, 7 volumes of ethanol (~750 g/l) TS, and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 20 mg of the test substance per ml, and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with iodine (0.1 mol/l) VS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 50 ml of a mixture of 7 volumes of acetic anhydride R and 3 volumes of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 44.99 mg of $C_{22}H_{23}NO_7$,HCl.

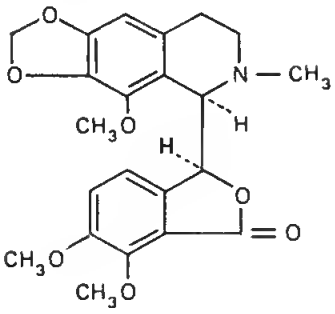
NOSCAPINUM

NOSCAPINE

Molecular formula. $C_{22}H_{23}NO_7$

Relative molecular mass. 413.4

Graphic formula.



Chemical name. Narcotine; (5*R*)-5-[(1*S*-6,7-dimethoxyphthalidyl]-5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinoline; [*S*-(*R**,*S**)]-6,7-dimethoxy-3-(5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)-1(3*H*)-isobenzofuranone; CAS Reg. No. 128-62-1.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; sparingly soluble in boiling water; slightly soluble in ethanol (~750 g/l) TS and ether R.

Category. Antitussive drug.

Storage. Noscapine should be kept in a well-closed container.

Requirements

Definition. Noscapine contains not less than 98.5% and not more than 101.0% of $C_{22}H_{23}NO_7$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from noscapine RS or with the *reference spectrum* of noscapine.

B. The absorption spectrum of a 0.050 mg/ml solution in methanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 291 nm and 310 nm and a minimum at about 263 nm. The ratio of the absorbance at 310 nm to that at 291 nm is about 1.2.

C. To 10 mg add about 0.5 ml of sulfuric acid (~1760 g/l) TS and mix; a greenish yellow solution is formed, which becomes red and finally violet on heating.

D. Melting temperature, about 175 °C.

Specific optical rotation. Use a 20 mg/ml solution in hydrochloric acid (0.1 mol/l) VS; $[\alpha]_D^{20\,°C} = +42$ to $+48°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 45 volumes of acetone R, 45 volumes of toluene R, 7 volumes of ethanol (~750 g/l) TS, and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with iodine (0.1 mol/l) VS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 40 ml of glacial acetic acid R1, warming gently, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 41.34 mg of $C_{22}H_{23}NO_7$.

NYSTATINUM

NYSTATIN

Chemical name. Nystatin; CAS Reg. No. 1400-61-9.

Description. A yellow to light brown powder.

Solubility. Very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS and methanol R; practically insoluble in ether R.

Category. Antifungal drug.

Storage. Nystatin should be kept in a tightly closed container, protected from light and stored at a temperature not exceeding 5 °C.

Additional information. Nystatin is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. The potency of Nystatin may fall at a rate of 1% per month when it is stored under the conditions mentioned above.

Requirements

Definition. Nystatin is a mixture of substances produced by the growth of certain strains of *Streptomyces noursei*, the main component of which is nystatin A_1.

Nystatin contains not less than 4400 International Units per mg, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.10 g in a mixture of 50 ml of methanol R and 5 ml of glacial acetic acid R, add sufficient methanol R to produce 100 ml, and dilute 1 ml to 100 ml with methanol R. The absorption spectrum of the resulting solution, when observed between 240 nm and 350 nm, exhibits three maxima at about 291 nm, 305 nm, and 319 nm; the ratio of the absorbance of a 1-cm layer at 291 nm to that at 305 nm is between 0.61 and 0.73, and the ratio of the absorbance at 319 nm to that at 305 nm is between 0.83 and 0.96.

B. Shake 30 mg with 5 ml of water for 2 minutes, add 2 ml of a solution prepared by dissolving 0.1 g of pyrogallol R in 100 ml of decolorized fuchsin TS, heat on a water-bath until a dark pink colour is produced, cool, and allow to stand for 1 hour; the pink colour is retained.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 50 mg/g.

pH value. Shake 0.3 g with 10 ml of carbon-dioxide-free water R; pH of the suspension, 6.5–8.0.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using Petri dishes or rectangular trays filled to a depth of 1–2 mm with culture medium Cm3 having a final pH of 6.0–6.2 and inoculated with *Saccharomyces cerevisiae* (NCYC 87; ATCC 9763) as the test organism, adding an appropriate concentration of nystatin (usually between 25 and 300 IU per ml) and incubating at a temperature of 29–33 °C. Prepare the solutions of the test substance by dissolving 75 mg in sufficient dimethylformamide R to produce 50 ml and dilute 10 ml to 200 ml with sterile phosphate buffer pH 6.0, TS3. Protect the solutions from light throughout the assay. The precision of the assay is such that the fiducial limits of error of the estimated potency (P = 0.95)

are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 4400 IU of nystatin per mg, calculated with reference to the dried substance.

OLEUM ARACHIDIS
ARACHIS OIL

Chemical name. Peanut oil; CAS Reg. No. 8002-03-7.

Other name. Peanut oil.

Description. A yellowish, clear, and viscous liquid; odourless, or with a very faint nut-like odour.

Miscibility. Very slightly miscible with ethanol (~750 g/l) TS; miscible with ether R, and light petroleum R.

Category. Oleaginous vehicle; solvent.

Storage. Arachis oil should be kept in a well-filled and well-closed container, protected from light, and stored in a cool place.

Additional information. The quality of the material described is not suitable for parenteral administration.

Requirements

Definition. Arachis oil is the refined fixed oil obtained from the seed kernels of *Arachis hypogaea* L.

Identity test
To 0.5 ml add 10 ml of potassium hydroxide/ethanol TS1 and, while shaking intermittently, heat in a water-bath at 80 °C for 15 minutes. Allow to stand for 1 hour; a cloudy, gelatinous mixture is formed which adheres to the walls of the tube.

Refractive index. $n_D^{20} = 1.468–1.472$.

Relative density. $d_{20}^{20} = 0.912–0.920$.

Acid value. Not more than 0.6.

Saponification value. 185–195.

Iodine value. 83–103.

Unsaponifiable matter. Not more than 15 mg/g.

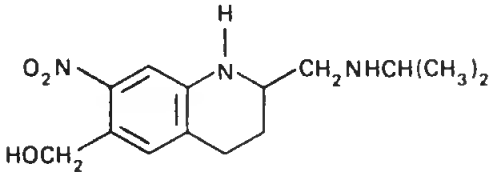
Peroxide value. Not more than 5.0.

OXAMNIQUINUM
OXAMNIQUINE

Molecular formula. $C_{14}H_{21}N_3O_3$

Relative molecular mass. 279.3

Graphic formula.



Chemical name. 1,2,3,4-Tetrahydro-2-[(isopropylamino)methyl]-7-nitro-6-quinolinemethanol; 1,2,3,4-tetrahydro-2-[[(1-methylethyl)amino]methyl]-7-nitro-6-quinolinemethanol; CAS Reg. No. 21738-42-1.

Description. A yellow-orange, crystalline powder.

Solubility. Sparingly soluble in water, soluble in methanol R and acetone R.

Category. Antischistosomal.

Storage. Oxamniquine should be kept in a well-closed container.

Requirements

Definition. Oxamniquine contains not less than 97.0% and not more than 103.0% of $C_{14}H_{21}N_3O_3$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from Oxamniquine RS or with the *reference spectrum* of oxamniquine.

B. To 10 mg add 10 mg of zinc R powder, 1.0 ml of water, and about 0.5 ml of hydrochloric acid (~250 g/l) TS. Heat in a water-bath for 5 minutes, cool in ice, add 1 ml of sodium nitrite (100 g/l) TS, and remove the excess nitrite by adding sufficient sulfamic acid (50 g/l) TS. Add 0.5 ml of the resulting solution to a mixture of 0.5 ml of 2-naphthol TS1 and 2.0 ml of sodium hydroxide (~80 g/l) TS; an orange-red colour is produced.

C. Melting temperature, about 151 °C.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 50 µg/g.

Iron. Ignite 1.0 g; cool and dissolve the residue in 5 ml of hydrochloric acid (~250 g/l) FeTS and 30 ml of water. Treat the solution as described under 2.2.4 Limit test for iron; the iron content is not more than 50 µg/g.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not more than 20 mg/g.

pH value. Shake 0.1 g with 10 ml of carbon-dioxide-free water R; pH of the suspension, 8.0–10.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and 20 volumes of chloroform R, 10 volumes of hexane R, 2 volumes of 2-propanol R, and 0.5 volume of isopropylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of equal volumes of chloroform R and methanol R containing (A) 25 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes until the solvents have evaporated. Examine the chromatogram in ultraviolet light first at 254 nm then at 365 nm. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, previously neutralized to oracet blue R/acetic acid TS. Titrate with per-

chloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.93 mg of $C_{14}H_{21}N_3O_3$.

OXYGENIUM
OXYGEN

O_2

Relative molecular mass. 32.00

Chemical name. Oxygen; CAS Reg. No. 7782-44-7.

Description. A colourless gas; odourless.

Solubility. One volume dissolves in about 32 volumes of water and in about 7 volumes of ethanol (~750 g/l) TS, both at a pressure of 101.3 kPa and 20 °C.

Category. Gas for inhalation.

Storage. Oxygen should be kept as compressed gas or liquid at cryogenic temperature, in appropriate containers complying with the safety regulations of the national authority. Valves or taps should not be lubricated with oil or grease.

Labelling. An ISO standard[1] requires that cylinders containing oxygen intended for medical use should bear the name of the contents in legible and permanent characters and, preferably, also the molecular formula O_2.

Additional information. In the analysis of medicinal gases certain tests are not intended for hospital pharmacists. They are solely applicable by laboratories equipped with the specialized apparatus.

Requirements

Oxygen contains not less than **99.5% v/v** of O_2.

- *Oxygen labelled as having been produced by the air-liquefaction process may be exempted from the requirements of the tests for carbon monoxide and carbon dioxide.*

[1] *International Standard 32. Gas cylinders for medical use – marking for identification of content.* International Organization for Standardization, Switzerland, 1977.

Identity tests

A. Place a glowing splinter of wood into the test gas; the splinter bursts into flame.

B. Shake with alkaline pyrogallol TS; the test gas is absorbed and the solution becomes dark brown (distinction from Dinitrogen oxide).

• *Note*: For the following tests deliver the test gas at a rate of 4 litres per hour.

Carbon monoxide

• Either test A or test B may be applied.

A. The apparatus (Fig. 7) consists of the following parts connected in series:

— a U-tube (U1) containing desiccant silica gel R impregnated with chromium trioxide R;

— a wash bottle (F1) containing 100 ml of potassium hydroxide (~400 g/l) TS;

— a U-tube (U2) containing pellets of potassium hydroxide R;

— a U-tube (U3) containing phosphorus pentoxide R dispersed on previously granulated, fused pumice;

— a U-tube (U4) containing 30 g of recrystallized iodine pentoxide R in granules, previously dried at 200 °C and kept at a temperature of 120 °C (T) during the test. The iodine pentoxide is packed in the tube in 1-cm columns separated by 1-cm columns of glass wool to give an effective length of 5 cm;

— a reaction tube (F2) containing 2.0 ml of potassium iodide (160 g/l) TS and 0.15 ml of starch TS.

Flush the apparatus with 5.0 litres of argon R. If necessary, discharge the blue colour in tube F2 containing potassium iodide (160 g/l) TS by adding a sufficient volume of freshly prepared sodium thiosulfate (0.002 mol/l) VS. Continue flushing with argon R until not more than 0.045 ml of sodium thiosulfate (0.002 mol/l) VS is required after the passage of 5.0 litres of argon R. Pass 7.5 litres of the test gas from the container through the apparatus. Flush the last traces of liberated iodine into the reaction tube by passing 1.0 litre of argon R through the apparatus. Titrate the liberated iodine with sodium thiosulfate (0.002 mol/l) VS. Repeat the procedure using 7.5 litres of argon R.

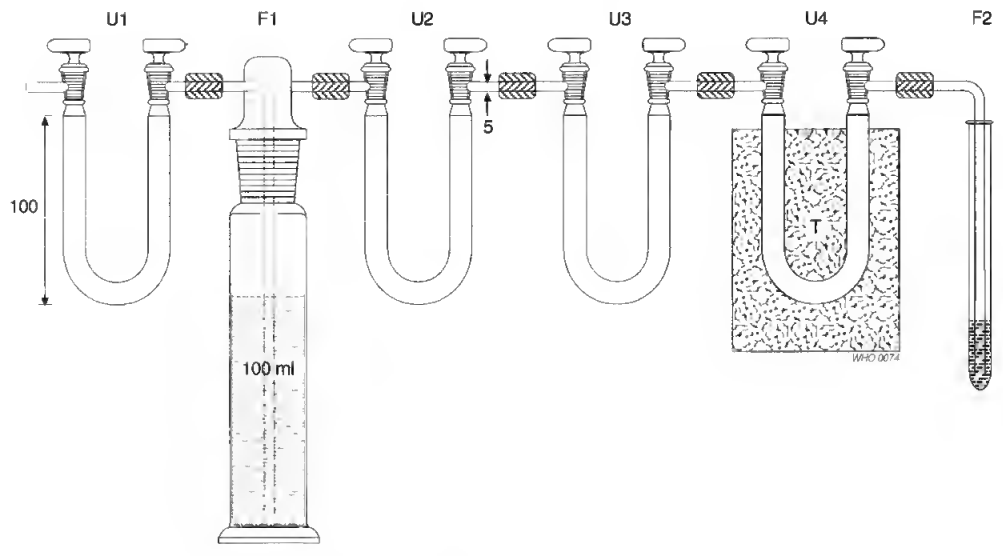


Figure 7. Apparatus for the determination of carbon monoxide in medicinal gases
Measurements in mm.
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B. Determine the content using a carbon monoxide detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the carbon monoxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 5 µl/l.

Note: For the following tests – "Carbon dioxide", "Oxidizing substances", and "Acidity and alkalinity" – pass the test gas through the appropriate reagent contained in a hermetically closed flat-bottomed glass cylinder (with dimensions such that 50 ml of liquid reaches a height of 12–14 cm) that is fitted with (a) a delivery tube terminated by a capillary 1 mm in internal diameter and placed within 2 mm of the bottom of the cylinder; and (b) an outlet tube.

Prepare the reference solutions in identical cylinders.

Carbon dioxide

- Either test A or test B may be applied.

A. Pass 1.0 litre of the test gas through 50 ml of a clear solution of barium hydroxide (0.15 mol/l) VS. Similarly prepare a reference solution by adding 1.0 ml of a 1.1 mg/ml solution of sodium hydrogen carbonate R in carbon-dioxidefree water R to 50 ml of barium hydroxide (0.15 mol/l) VS.

Any turbidity in the solution after the passage of the test gas is not more intense than that of the reference solution (300 µl/l).

B. Determine the content using a carbon dioxide detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the carbon dioxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 300 µl/l.

Oxidizing substances. To two cylinders add 50 ml of freshly prepared potassium iodide/starch TS1 and about 0.2 ml of glacial acetic acid R. Protect the cylinders from light. Pass 5.0 litres of the test gas into one of the solutions and compare the colour produced.

The solutions in both cylinders remain colourless.

Water

- Either test A or test B may be applied.

A. The apparatus consists either of an electrolytic hygrometer as described below, an appropriate humidity detector tube, or a capacity hygrometer.

The measuring cell consists of a thin film of phosphoric anhydride placed between two coiled platinum wires that act as electrodes. The water vapour in the test gas is absorbed by the phosphoric anhydride to form phosphoric acid, which acts as an electrical conductor.

Before introducing the test gas into the device, allow the gas to stabilize at room temperature and make sure that the temperature is constant throughout the apparatus. Apply a continuous voltage across the electrodes to produce electrolysis of the water and regeneration of phosphoric anhydride. Measure the resulting electrical current, which is proportional to the water

content in the test gas. (This is a self-calibrating system that obeys Faraday's law.)

Calculate the content of water; not more than 60 µg/l.

B. Determine the content using a water vapour detector tube. Pass the required volume of the test gas through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of the test gas to purge the tubing to an appropriate flow. Fit the water vapour detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of the test gas through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 60 µl/l.

Acidity and alkalinity. Pass 2.0 litres of the test gas through a mixture of 0.10 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

For *reference solution 1*, use 50 ml of carbon-dioxide-free water R. For *reference solution 2*, use a mixture of 0.20 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

To each solution add 0.1 ml of methyl red/ethanol TS; the intensity of the colour in the solution of the test gas is between those of reference solutions 1 and 2.

Assay

- Either method A or method B may be applied.

A. For the determination use a 25-ml capacity gas burette (Fig. 8) in the form of a chamber with at its upper end, a tube graduated in 0.2% between 95 and 100, and isolated at each end by a tap with a conical barrel. The lower tap is joined to a tube with an olive-shaped nozzle and is used to introduce the test gas into the apparatus. A cylindrical funnel above the upper tap is used to introduce the absorbent solution. Wash the burette with water and dry. Open the two taps. Connect the nozzle to the container of the test gas and set the flow rate to 1 litre per minute. Flush the burette by passing the gas through it for 1 minute. Close the upper tap of the burette and immediately afterwards the lower tap. Rapidly disconnect the burette from the container of the test gas, and give a half turn to the upper tap to eliminate any excess pressure in the burette. Keeping the burette vertical, fill the funnel

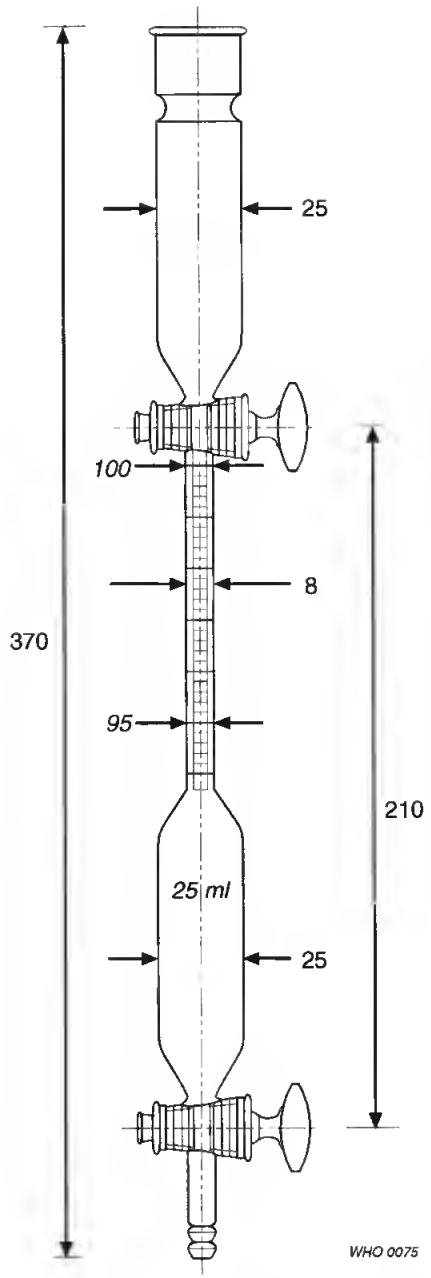

Figure 8. Burette used for the assay of oxygen Measurements in mm.
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with a freshly prepared mixture of 21 ml of potassium hydroxide (~560 g/l) TS and 130 ml of sodium dithionite (200 g/l) TS. Open the upper tap slowly. The solution absorbs the oxygen and enters the burette. Allow to stand for 10 minutes without shaking.

Read the level of the liquid meniscus on the graduated part of the burette; the figure represents the content of oxygen as a percentage in v/v.

B. Oxygen in medicinal gases can also be determined using a paramagnetic analyser, which measures electronically the molecule's interaction with magnetic fields.

Carry out the method according to the instrument manufacturer's instructions.

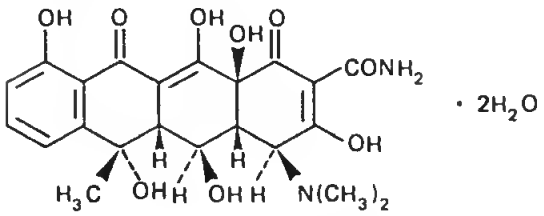
OXYTETRACYCLINI DIHYDRAS
OXYTETRACYCLINE DIHYDRATE

Oxytetracycline dihydrate (non-injectable)
Oxytetracycline dihydrate, sterile

Molecular formula. $C_{22}H_{24}N_2O_9,2H_2O$

Relative molecular mass. 496.5

Graphic formula.



Chemical name. (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide dihydrate; [4*S*-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2naphthacenecarboxamide dihydrate; CAS Reg. No. 6153-64-6 (dihydrate).

Description. A pale yellow, crystalline powder; odourless.

Solubility. Very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; freely soluble in dilute acids and alkalis.

Category. Antibacterial drug.

Storage. Oxytetracycline dihydrate should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Oxytetracycline dihydrate indicates that the substance complies with the additional requirements for sterile Oxytetracycline dihydrate and may be used for parenteral administration or for other sterile applications.

Additional information. Oxytetracycline dihydrate darkens on exposure to strong sunlight. It deteriorates in solutions having a pH below 2 and is rapidly destroyed by alkali hydroxide solutions.

Requirements

Definition. Oxytetracycline dihydrate contains not less than 920 International Units of Oxytetracycline per mg, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a kieselguhr coating prepared as follows: To 25 g of kieselguhr R1 add 50 ml of a mixture of 2.5 ml of glycerol R and 47.5 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS. Coat the plates with this mixture and allow them to dry at room temperature for about 70–90 minutes, or until sufficiently dry to give a satisfactory separation. As the mobile phase, take 200 ml of a mixture of 2 volumes of ethyl acetate R, 2 volumes of chloroform R, and 1 volume of acetone R, shake with 25 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS, allow to settle, and use the lower layer. Apply separately to the plate 1 µl of each of 3 solutions in methanol R containing (A) 0.50 mg of the test substance per ml, (B) 0.50 mg of Oxytetracycline dihydrate RS per ml, and (C) a mixture of 0.50 mg of chlortetracycline hydrochloride RS per ml, 0.50 mg of Oxytetracycline dihydrate RS per ml, and 0.50 mg of tetracycline hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows 3 clearly separated spots.

B. To about 1 mg add 2 ml of sulfuric acid (~1760 g/l) TS; a deep red colour is produced, which changes to yellow on the addition of 0.1 ml of water.

Specific optical rotation. Dissolve 0.25 g in sufficient hydrochloric acid (0.1 mol/l) VS to produce 25 ml, and allow to stand for 60 minutes. Measure the rotation and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = –203 to –216 °.

Sulfated ash. Not more than 5.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not less than 40 mg/g and not more than 75 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 4.5–7.5.

Absorption in the ultraviolet region. The absorption spectrum of a 20 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 400 nm, exhibits 2 maxima at about 268 nm and 353 nm. The absorbance of a 1-cm layer at 353 nm is not less than 0.54 and not more than 0.58.

Light-absorbing impurities. Prepare a 2.0 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and measure within 1 hour of preparation the absorbance of a 1-cm layer at 430 nm; the absorbance does not exceed 0.25. Prepare a 10 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, and measure within 1 hour of preparation the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.20.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5–6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 2 and 20 IU per ml), and an incubation temperature of 37–39 °C, or (*b*) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9–6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 0.5 and 2 IU per ml), and an incubation temperature of 30–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 900 IU of oxytetracycline per mg, calculated with reference to the anhydrous substance.

Additional Requirements for Oxytetracycline Dihydrate for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.4 IU of endotoxin RS per mg of oxytetracycline.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using a diluting fluid containing 1 ml of macrogol *p*-isooctylphenyl ether R per litre of peptone (1 g/l) TS. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

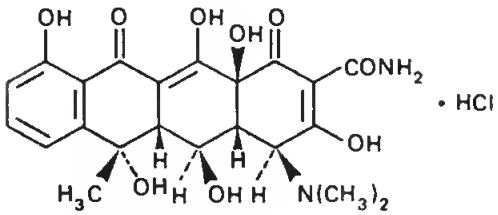
OXYTETRACYCLINI HYDROCHLORIDUM
OXYTETRACYCLINE HYDROCHLORIDE

Oxytetracycline hydrochloride (non-injectable)
Oxytetracycline hydrochloride, sterile

Molecular formula. $C_{22}H_{24}N_2O_9,HCl$

Relative molecular mass. 496.9

Graphic formula.



Chemical name. (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; [4*S*-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; CAS Reg. No. 2058-46-0.

Description. A yellow, crystalline powder; odourless.

Solubility. Soluble in 2 parts of water and in 45 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Oxytetracycline hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Oxytetracycline hydrochloride indicates that the substance complies with the additional requirements for sterile Oxytetracycline hydrochloride and may be used for parenteral administration or for other sterile applications.

Additional information. Oxytetracycline hydrochloride is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Dissolved in water it becomes turbid on standing owing to the separation of the base caused

through partial hydrolysis of the hydrochloride. It deteriorates in solutions of pH below 2, and is rapidly destroyed by alkali hydroxide solutions.

Requirements

Definition. Oxytetracycline hydrochloride contains not less than 870 International Units of Oxytetracycline per mg, calculated with reference to the anhydrous substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a kieselguhr coating prepared as follows: To 25 g of kieselguhr R1 add 50 ml of a mixture of 2.5 ml of glycerol R and 47.5 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS. Coat the plates with this mixture, and allow them to dry at room temperature for about 70–90 minutes, or until sufficiently dry to give a satisfactory separation. As the mobile phase, take 200 ml of a mixture of 2 volumes of ethyl acetate R, 2 volumes of chloroform R, and 1 volume of acetone R, shake with 25 ml of disodium edetate (0.1 mol/l) VS previously adjusted to pH 7 with ammonia (~100 g/l) TS, allow to settle, and use the lower layer. Apply separately to the plate 1 µl of each of 3 solutions in methanol R containing (A) 0.50 mg of the test substance per ml, (B) 0.50 mg of Oxytetracycline hydrochloride RS per ml, and (C) a mixture of 0.50 mg of chlortetracycline hydrochloride RS per ml, 0.50 mg of Oxytetracycline hydrochloride RS per ml, and 0.50 mg of tetracycline hydrochloride RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows 3 clearly separated spots.

B. To about 1 mg add 2 ml of sulfuric acid (~1760 g/l) TS; a deep red colour is produced, which changes to yellow on the addition of 0.1 ml of water.

C. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Dissolve 0.25 g in sufficient hydrochloric acid (0.1 mol/l) VS to produce 25 ml and allow to stand for 60 minutes. Measure the rotation and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = −188 to −200 °.

Sulfated ash. Not more than 5.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.25 g of the substance; the water content is not more than 20 mg/g.

pH value. pH of a 10 mg/ml solution, 2.0–3.0.

Absorption in the ultraviolet region. The absorption spectrum of a 20 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 400 nm, exhibits 2 maxima at about 268 nm and 353 nm. The absorbance of a 1-cm layer at 353 nm is not less than 0.54 and not more than 0.58.

Light-absorbing impurities. Prepare a 2.0 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and measure within 1 hour of preparation the absorbance of a 1-cm layer at 430 nm; the absorbance does not exceed 0.50.

Prepare a 10 mg/ml solution in a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R and measure within 1 hour of preparation the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.20.

Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5–6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 2 and 20 IU per ml), and an incubation temperature of 37–39 °C, or (*b*) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9–6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of oxytetracycline (usually between 0.5 and 2 IU), and an incubation temperature of 30–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 870 IU of oxytetracycline per mg, calculated with reference to the anhydrous substance.

Additional Requirements for Oxytetracycline Hydrochloride for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.4 IU of endotoxin RS per mg of oxytetracycline.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane nitration test procedure. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Basic tests for drugs:
pharmaceutical substances, medicinal plant materials and dosage forms
1998 (94 pages)

Basic tests for pharmaceutical dosage forms
1991 (134 pages)

Quality Assurance of Pharmaceuticals: a compendium of guidelines and related materials
Volume 1: 1997 (244 pages)
Volume 2: good manufacturing practices and inspection. 2004 (236 pages)

WHO Expert Committee on Specifications for Pharmaceutical Preparations
Fortieth report
WHO Technical Report Series, No. 937,
2006 (461 pages)

International nonproprietary names (INN) for pharmaceutical substances
Cumulative list no. 11
2004 (available in CD-ROM format only)

The use of essential medicines
Report of the WHO Expert Committee
(including the 13th Model List of Essential Medicines)
WHO Technical Report Series, No. 920,
2004 (133 pages)

WHO Expert Committee on Biological Standardization
Fifty-fourth report
WHO Technical Report Series, No. 927,
2005 (160 pages)

Further information on these or other WHO publications can be obtained from WHO Press, World Health Organization, 1211 Geneva 27, Switzerland.
Attention is also drawn to the WHO Medicines web site
(http://www.who.int/medicines)

The International Pharmacopoeia

The International Pharmacopoeia (Ph. Int.) constitutes a collection of recommended procedures for analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms that is intended to serve as source material for reference or adaptation by any WHO Member State wishing to establish pharmaceutical requirements. The pharmacopoeia, or any part of it, shall have legal status, whenever a national or regional authority expressly introduces it into appropriate legislation. Further explanation of the role of *The International Pharmacopoeia* is provided in the paragraphs entitled "Scope and function" at the end of the Preface to this edition.

This is the fourth edition of *The International Pharmacopoeia*. It comprises two volumes; the General Notices and monographs for pharmaceutical substances (A to O) are to be found in Volume 1 and the remaining monographs for pharmaceutical substances together with those for dosage forms and radiopharmaceutical preparations, the methods of analysis and the reagent sections are to be found in Volume 2.

World Health Organization

ISBN 92 4 156401 X

The International Pharmacopoeia

Fourth edition

Volume 2

World Health Organization

Geneva
2006

The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization's priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO's Member countries and the collaboration of world leaders in public health and the biomedical sciences.

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The International Pharmacopoeia

FOURTH EDITION

Pharmacopoea Internationalis
Editio Quarta

Volume 2

World Health Organization
Geneva
2006

WHO Library Cataloguing-in-Publication Data

The International pharmacopoeia = Pharmacopoea internationalis. – 4th ed.

2 v.

1.Pharmacopoeias 2.Pharmaceutical preparations – standards 3.Pharmaceutical preparations – analysis 4.Dosage forms – standards I.World Health Organization.

ISBN 92 4 156301 X (LC/NLM classification: QV 738.1)

Printed in Singapore

Contents

Monographs

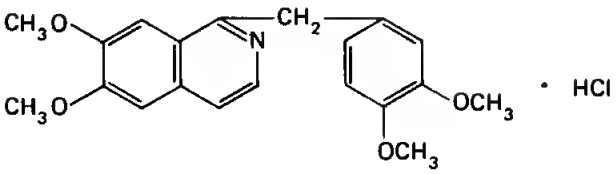
Pharmaceutical substances

PAPAVERINI HYDROCHLORIDUM
PAPAVERINE HYDROCHLORIDE

Molecular formula. $C_{20}H_{21}NO_4,HCl$

Relative molecular mass. 375.9

Graphic formula.



Chemical name. 6,7-Dimethoxy-1-veratrylisoquinoline hydrochloride; 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline hydrochloride; CAS Reg. No. 61-25-6.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Sparingly soluble in water; soluble in 120 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Spasmolytic.

Storage. Papaverine hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Papaverine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{20}H_{21}NO_4,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from papaverine hydrochloride RS or with the *reference spectrum* of papaverine hydrochloride.

B. Treat about 10 mg with 3 ml of acetic anhydride R, add cautiously 3 drops of sulfuric acid (~1760 g/l) TS and heat on a water-bath for 3–4 minutes; a yellow colour with a green fluorescence is produced.

C. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

D. Dissolve 20 mg in 10 ml of water, add drop by drop ammonia (~100 g/l) TS, and set aside. Filter, wash the precipitate with water, and dry at 105 °C; melting temperature, about 146 °C (papaverine base).

Clarity and colour of solution. A solution of 0.20 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Gn2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 20 mg/ml solution, 3.0–4.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 7 volumes of toluene R, 2 volumes of ethyl acetate R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 4 volumes of hydrochloric acid (0.01 mol/l) VS and 1 volume of ethanol (~750 g/l) TS containing (A) 0.050 g of the test substance per ml and (B) 0.50 mg of codeine R per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air until the odour of diethylamine is no longer perceptible, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

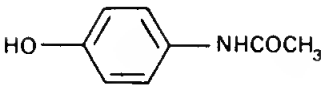
Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 37.59 mg of $C_{20}H_{21}NO_4$,HCl.

PARACETAMOLUM
PARACETAMOL

Molecular formula. $C_8H_9NO_2$

Relative molecular mass. 151.2

Graphic formula.



Chemical name. 4′-Hydroxyacetanilide; *N*-(4-hydroxyphenyl)acetamide; CAS Reg. No. 103-90-2.

Other name. Acetaminophen.

Description. A white, crystalline powder; odourless.

Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/l) TS and acetone R; practically insoluble in ether R.

Category. Analgesic; antipyretic.

Storage. Paracetamol should be kept in a tightly closed container, protected from light.

Requirements

Definition. Paracetamol contains not less than 98.5% and not more than 101.0% of $C_8H_9NO_2$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.05 g in 100 ml of methanol R. To 1 ml of this solution add 0.5 ml of hydrochloric acid (0.1 mol/l) VS and dilute to 100 ml with methanol R. Protect the solution from light and immediately measure the absorbance of a 1-cm layer at the maximum wavelength of about 249 nm; about 0.88.

B. Dissolve 0.1 g in 10 ml of water and add 0.05 ml of ferric chloride (25 g/l) TS; a violet-blue colour is produced.

C. Boil 0.1 g with 1 ml of hydrochloric acid (~70 g/l) TS for 3 minutes, add 10 ml of water and cool; no precipitate is formed. Add 0.05 ml of potassium

dichromate (0.0167 mol/l) VS; a violet colour, which does not turn to red (distinction from phenacetin) is slowly produced.

Melting range. 168–172 °C.

Heavy metals. Use 1.0 g and a mixture of 85 volumes of acetone R and 15 volumes of water for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

4-Aminophenol. Dissolve 0.5 g in a mixture of equal volumes of methanol R and water and dilute to 10 ml with this solvent mixture. Add 0.2 ml of alkaline sodium nitroprusside TS, mix, and allow to stand for 30 minutes. Prepare similarly a reference solution containing 0.5 g of 4-aminophenol-free paracetamol R and 0.5 ml of a solution containing 0.050 mg/ml of 4-aminophenol R in the same solvent mixture. The colour of the test solution is not more intense than that of the reference solution (0.05 mg/g).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 65 volumes of chloroform R, 25 volumes of acetone R, and 10 volumes of toluene R as the mobile phase. Allow the solvent front to ascend 14 cm above the line of application, using an unlined chromatographic chamber. Prepare the following 4 test solutions: For solution (A) transfer 1.0 g of finely powdered substance to be examined to a glass-stoppered tube, add 5 ml of ether R, and shake mechanically for 30 minutes. Centrifuge the tube until a clear supernatant liquid is obtained and separate this from the solid. For solution (B) dilute 1 ml of solution A to 10 ml with ethanol (~750 g/l) TS. For solution (C) dissolve 25 mg of 4-chloroacetanilide R in 50 ml of ethanol (~750 g/l) TS. For solution (D) dissolve 0.25 g of 4-chloroacetanilide R and 0.1 g of the substance to be examined in sufficient ethanol (~750 g/l) TS to produce 100 ml. Apply separately to the plate 200 µl of solution A and 40 µl of each of the remaining 3 solutions. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air and examine the chromatogram in ultraviolet light (254 nm). Any spot due to 4-chloroacetanilide obtained with solution A is not more intense than the corresponding spot obtained with solution C. Any spot obtained with solution B, other than the principal spot and the spot corresponding to 4-chloroacetanilide, is not more intense than the spot obtained with solution C. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots corresponding to 4-chloroacetanilide and the substance being examined, the latter having a lower R_f value.

Assay. Transfer about 0.25 g, accurately weighed, to a flask, add 10 ml of hydrochloric acid (~70 g/l) TS and boil under a reflux condenser for 1 hour. Wash the condenser with 30 ml of water, add 1 g of potassium bromide R to the combined solution, and proceed as described under 2.7 Nitrite titration, titrating with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 15.12 mg of $C_8H_9NO_2$.

PARAFFINUM ALBUM
WHITE, SOFT PARAFFIN

PARAFFINUM FLAVUM
YELLOW SOFT PARAFFIN

Chemical name. White and yellow petrolatum.

Other names. White petrolatum, yellow petrolatum; vaselinum album, vaselinum flavum.

Description. A white or a pale yellow to yellow, soft, unctuous mass; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in ether R, and in most fixed and volatile oils.

Category. Ointment base.

Storage. White and yellow soft paraffins should be kept in a well-closed container.

Additional information. In a thin layer or when melted, both paraffins show a slight fluorescence. Melting point, within 38–60 °C.

Requirements

Definition. White and yellow soft paraffins are purified mixtures of semi-solid hydrocarbons obtained from petroleum. White soft paraffin is bleached. To prevent oil separation, soft paraffins may contain a suitable stabilizer.

Identity tests

A. Melt 2 g until a homogeneous mass is obtained and immediately add 2 ml of water and 0.2 ml of iodine (0.1 mol/l) VS. Heat; as soon as two liquid phases are obtained, shake and cool; the upper solid phase should have a pinkish violet colour.

B. Heat a small quantity of either White soft paraffin or Yellow soft paraffin and ignite; a luminous flame is observed and a deposit of carbon is formed.

Sulfated ash. Not more than 1.0 mg/g.

Alkalinity. To 35 g add 100 ml of boiling water, cover the beaker, and, while stirring, heat to boiling for 5 minutes. Allow the phases to separate, transfer the aqueous layer to a suitable dish, and wash the paraffin with two portions, each of 50 ml, of boiling water which are added to the dish. Add 1 drop of phenolphthalein/ethanol TS and boil; the colour does not change to pink. (Keep this solution for "Acidity".)

Acidity. To the above solution, add 0.1 ml of methyl orange/ethanol TS; the colour does not change to red or pink.

Organic acids. To 20 g add 100 ml of a mixture of equal volumes of neutralized ethanol TS and water, mix thoroughly, and heat to boiling. Add 1 ml of phenolphthalein/ethanol TS and titrate rapidly with carbonate-free sodium hydroxide (0.1 mol/l) VS to a sharp pink endpoint, the colour change being observed in the ethanol-water layer; not more than 0.4 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is required.

Fixed oils, fats, and rosin. Digest 10 g with 50 ml of sodium hydroxide (~200 g/l) TS at 100 °C for 30 minutes. Separate the aqueous layer and acidify with sulfuric acid (~570 g/l) TS; the remaining phase does not show any oil or solid matter.

Ultraviolet absorption. Dissolve 50 mg in 100 ml of 2,2,4-trimethylpentane R. Measure the absorbance of a 1-cm layer at about 290 nm. White soft paraffin does not exceed 0.5; yellow soft paraffin does not exceed 0.75.

PARAFFINUM DURUM
HARD PARAFFIN

Chemical name. Paraffin wax; paraffin waxes and hydrocarbon waxes; CAS Reg. No. 8002-74-2.

Description. A colourless or white, slightly unctuous mass showing a crystalline structure; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; freely soluble in ether R.

Category. Ointment base; viscosity-increasing agent.

Storage. Hard paraffin should be kept in a well-closed container.

Additional information. Congealing point, within 47–65 °C.

Requirements

Definition. Hard paraffin is a purified mixture of solid hydrocarbons obtained from petroleum.

Identity tests

A. Melt 2 g until a homogeneous mass is obtained and immediately add 2 ml of water and 0.2 ml of iodine (0.1 mol/l) VS. Heat; as soon as two liquid phases are obtained, shake and cool; the upper solid phase has a pinkish violet colour.

B. Heat a small quantity of Hard paraffin and ignite; a luminous flame is observed and a deposit of carbon is formed.

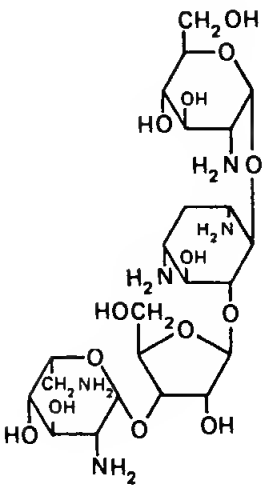
Sulfated ash. Not more than 1.0 mg/g.

Acidity or alkalinity. Boil 5 g with 10 ml of ethanol (~710 g/l) TS previously neutralized to litmus TS, cool, and add a few drops of litmus TS; the solution is neutral (violet).

PAROMOMYCINI SULFAS
PAROMOMYCIN SULFATE

Molecular formula. $C_{23}H_{45}N_5O_{14},xH_2SO_4$

Graphic formula.



Chemical name. *O*-2,6-Diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-*O*-β-D-ribofuranosyl-(1→5)-*O*-[2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxystreptamine sulfate (salt); *O*-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[*O*-2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine sulfate (salt); CAS Reg. No. 1263-89-4.

Description. A creamy white to light yellow powder; odourless or almost odourless.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Antiamoebic drug.

Storage. Paromomycin sulfate should be kept in a tightly closed container, protected from light.

Additional information. Paromomycin sulfate is very hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Paromomycin sulfate contains not less than 675 International Units of paromomycin per mg, calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a freshly prepared ammonium acetate (40 g/l) TS as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions containing (A) 20 mg of the test substance per ml and (B) 20 mg of paromomycin sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes, heat it at 105 °C for 1 hour, and spray it with triketohydrindene/butanol TS. Heat it again at 105 °C for 5 minutes and examine the chromatogram in daylight. The principal red spot obtained with solution A corresponds in position and appearance with that obtained with solution B.

B. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +50 to +55 °.

Sulfated ash. Not more than 20 mg/g.

Loss on drying. Dry to constant weight at 50 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 50 mg/g.

pH value. pH of a 30 mg/ml solution in carbon-dioxide-free water R, 5.0–7.5.

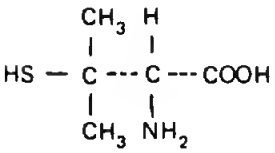
Assay. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus subtilis* (NCTC 10400) as the test organism, culture medium Cm1 with a final pH of 8.0, sterile phosphate buffer pH 7.8, TS, an appropriate concentration of paromomycin (usually between 1 and 4 IU per ml), and an incubation temperature of 37–39 °C; or (*b*) *Bacillus subtilis* (ATCC 6633) as the test organism, culture medium Cm1 with a final pH of 7.8, sterile phosphate buffer pH 8.0, TS1 or TS2, an appropriate concentration of paromomycin (usually between 2 and 8 IU per ml), and an incubation temperature of 36–38 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 675 IU of paromomycin per mg, calculated with reference to the dried substance.

PENICILLAMINUM
PENICILLAMINE

Molecular formula. $C_5H_{11}NO_2S$

Relative molecular mass. 149.2

Graphic formula.



Chemical name. 3-Mercapto-D-valine; 3,3-dimethyl-D-cysteine; CAS Reg. No. 52-67-5.

Description. A white or almost white, crystalline powder; odour, characteristic.

Solubility. Soluble in 9 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in chloroform R and ether R.

Category. Antidote.

Storage. Penicillamine should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Penicillamine is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Penicillamine contains not less than 95.0% and not more than 100.5% of $C_5H_{11}NO_2S$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 20 mg in 4 ml of water, add 2 ml of phosphotungstic acid TS and allow to stand for a few minutes; a deep blue colour is produced.

B. Dissolve 20 mg in 5 ml of water, add 0.05 ml of sodium hydroxide (~200 g/l) TS and 20 mg of triketohydrindene hydrate R; an intense blue or violet-blue colour is produced immediately.

Specific optical rotation. Use a 50 mg/ml solution in sodium hydroxide (1 mol/l) VS; $[\alpha]_D^{20\,°C} = -58$ to $-68°$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5 mg/g.

pH value. pH of a 10 mg/ml solution, 4.0–6.0.

Mercury

- The operations described below must be carried out in subdued light.

Transfer about 0.5 g, accurately weighed, to a 650-ml long-necked flask containing a few glass beads, incline the flask at an angle of about 45°, and add 2.5 ml of nitric acid (~1000 g/l) TS through a small funnel placed in the mouth of the flask. Allow the mixture to stand at room temperature until nitrous oxide fumes are evolved and the vigorous reaction subsides (5–30 minutes). Add 2.5 ml of sulfuric acid (~1760 g/l) TS through the funnel and heat, gently at first and then to the production of fumes of sulfur trioxide. Cool, then add cautiously 2.5 ml of nitric acid (~1000 g/l) TS, heat again to the production of sulfur trioxide fumes, and cool. Repeat this treatment once more, then add 50 ml of water, rinsing the funnel, and collecting the rinsings in the flask. Remove the funnel, boil the solution down to approximately half its volume (about 25 ml), and cool to room temperature. Transfer to a 250-ml separating funnel with the

aid of water and dilute with water to 50 ml. Add 1 ml of disodium edetate (20 g/l) TS and 1 ml of glacial acetic acid R and extract with small portions of chloroform R until the last chloroform extract remains colourless. Discard the chloroform extract and add 50 ml of sulfuric acid (0.125 mol/l) VS, 90 ml of water, and 10 ml of hydroxylamine hydrochloride (200 g/l) TS. Add dithizone standard TS, in portions of 0.3–0.5 ml, from a 10-ml burette. After each addition, shake the mixture well, allow the chloroform layer to separate, and discard it. Continue until an addition of dithizone standard TS remains green after shaking.

From the volume of the dithizone standard TS used, calculate the amount of mercury present in the test substance. It contains not more than 20 µg of Hg per g.

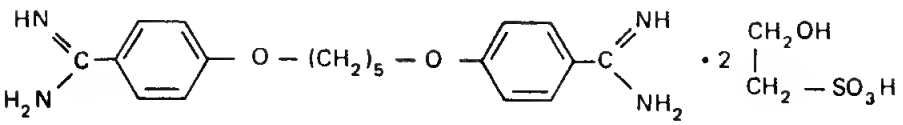
Assay. Dissolve about 0.1 g, accurately weighed, in 50 ml of water, add 5 ml of sodium hydroxide (1 mol/l) VS and 0.2 ml of dithizone TS and titrate with mercuric nitrate (0.02 mol/l) VS. Each ml of mercuric nitrate (0.02 mol/l) VS is equivalent to 5.968 mg of $C_5H_{11}NO_2S$.

PENTAMIDINI ISETIONAS
PENTAMIDINE ISETIONATE

Molecular formula. $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$

Relative molecular mass. 592.7

Graphic formula.



Chemical name. 4,4′-(Pentamethylenedioxy)dibenzamidine bis(2-hydroxyethanesulfonate); 4,4′-[1,5-pentanediylbis(oxy)]bis[benzenecarboximidamide]-bis(2-hydroxyethanesulfonate); CAS Reg. No. 140-64-7.

Description. A white, or almost white, crystalline powder; odourless.

Solubility. Soluble in 10 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in chloroform R and ether R.

Category. Antitrypanosomal drug; antileishmaniasis drug.

Storage. Pentamidine isetionate should be kept in a well-closed container.

Additional information. Pentamidine isetionate is hygroscopic.

Requirements

Definition. Pentamidine isetionate contains not less than 98.5% and not more than 102.5% of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 262 nm; the absorbance of a 1-cm layer at this wavelength is about 0.47.

B. To 0.5 g add 5 ml of water and heat to 80 °C to dissolve. Add 10 ml of sodium hydroxide (~50 g/l) TS, cool in ice, and filter. To 2 ml of the nitrate add 0.2 ml of nitric acid (~1000 g/l) TS followed by 0.2 ml of ceric ammonium nitrate TS; a red-orange colour is produced.

C. Melting temperature, about 190 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 40 mg/g.

pH value. pH of a 0.05 g/ml solution, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6, activated at 105 °C for 1 hour, as the coating substance (a precoated plate from a commercial source is suitable), and as the mobile phase the upper layer obtained by shaking together 10 volumes of water, 8 volumes of 1-butanol R, and 2 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

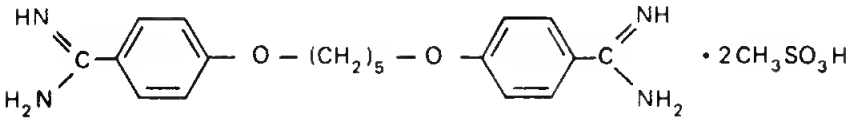
Assay. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.4 g of the test substance, accurately weighed, and 9 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 14.82 mg of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$.

PENTAMIDINI MESILAS
PENTAMIDINE MESILATE

Molecular formula. $C_{19}H_{24}N_4O_2,2CH_4O_3S$

Relative molecular mass. 532.6

Graphic formula.



Chemical name. 4,4′-(Pentamethylenedioxy)dibenzamidine dimethanesulfonate; 4,4′-[1,5-pentanediylbis(oxy)]bis[benzenecarboximidamide] dimethanesulfonate; CAS Reg. No. 6823-79-6.

Description. A white or light pink, granular powder; almost odourless.

Solubility. Slightly soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R, acetone R, and chloroform R.

Category. Antitrypanosomal drug; antileishmaniasis drug.

Storage. Pentamidine mesilate should be kept in a well-closed container.

Requirements

Definition. Pentamidine mesilate contains not less than 98.5% and not more than 102.5% of $C_{19}H_{24}N_4O_2,2CH_4O_3S$, calculated with reference to the dried substance.

Manufacture. The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Identity tests

A. To 0.5 g add 5 ml of water and heat to 80 °C to dissolve. Add 10 ml of sodium hydroxide (~50 g/l) TS, cool in ice, and filter. To 2 ml of the filtrate add 0.2 ml of nitric acid (~1000 g/l) TS followed by 0.2 ml of ceric ammonium nitrate TS; a yellow colour is produced.

B. Heat 0.5 g with 1 ml of sodium hydroxide (~400 g/l) TS in a test-tube; ammonia, perceptible by its odour, is evolved.

C. Dissolve 1 g in 10 ml of water at 80 °C, add 10 ml of sodium hydroxide (~50 g/l) TS, cool in ice, filter (keep the filtrate for test D), wash with 10 ml of water, and dry the precipitate at 105 °C; melting temperature, about 188 °C.

D. Transfer 10 ml of the filtrate obtained in test C to a platinum crucible, add 2.5 ml of hydrogen peroxide (~60 g/l) TS, mix with the help of a glass rod and evaporate to dryness on a water-bath. Dissolve the residue in 1 ml of water, add 1 ml of glacial acetic acid R and again evaporate to dryness on a water-bath, then ignite until free from carbon. Cool, mix the residue with 5 ml of water and filter, if necessary. Neutralize with hydrochloric acid (~70 g/l) TS and add an excess of 3 ml. Boil for 30 seconds, cool and proceed with reaction A described under 2.1 General identification tests as characteristic of sulfates.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

pH value. pH of a 0.05 g/ml solution prepared in warm water and then cooled, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6, activated at 105 °C for 1 hour, as the coating substance (a precoated plate from a commercial source is suitable), and as the mobile phase the upper layer obtained by shaking together 10 volumes of water, 8 volumes of 1-butanol R, and 2 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml (warm, if necessary) and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

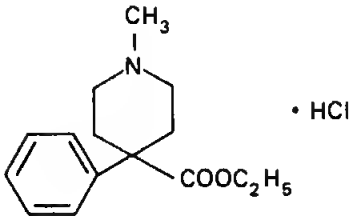
Assay. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.4 g, accurately weighed, and 9 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 13.32 mg of $C_{19}H_{24}N_4O_2,2CH_4O_3S$.

PETHIDINI HYDROCHLORIDUM
PETHIDINE HYDROCHLORIDE

Molecular formula. $C_{15}H_{21}NO_2,HCl$

Relative molecular mass. 283.8

Graphic formula.



Chemical name. Ethyl 1-methyl-4-phenylisonipecotate hydrochloride; ethyl 1-methyl-4-phenyl-4-piperidinecarboxylate hydrochloride; CAS Reg. No. 50-13-5.

Other name. Meperidine hydrochloride.

Description. A white, crystalline powder; odourless.

Solubility. Very soluble in water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Narcotic analgesic.

Storage. Pethidine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Pethidine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Pethidine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{15}H_{21}NO_2,HCl$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 0.5 ml of water, add about 0.1 ml of formaldehyde TS and 2 ml of sulfuric acid (~1760 g/l) TS; an orange-red colour is produced.

B. Dissolve 0.1 g in 5 ml of ethanol (~750 g/l) TS and add 15 ml of trinitrophenol (7 g/l) TS; a yellow, crystalline precipitate is produced. Collect the precipitate on a filter, wash with water, and dry at 105 °C; melting temperature, about 190 °C (picrate). (Keep a portion of the picrate for test C.)

C. Mix 5 mg of the picrate obtained in test B with 5 mg of the substance being examined; melting temperature, not less than 20 °C lower than that of the picrate alone.

D. A 10 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Melting range. After drying to constant weight at 105 °C, 187–190 °C.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.05 g/ml solution, 4.0–6.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of 2-phenoxyethanol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of a least 15 cm, remove the plate from the chromatographic chamber and allow the acetone to evaporate. Use the impregnated plate immediately, carrying out the chromatography in the same direction as the impregnation. Prepare the mobile phase by shaking together 1 volume of diethylamine R, 100 volumes of light petroleum R1, and 8 volumes of 2-phenoxyethanol R, allow to settle, and use the supernatant liquid. For the preparation of the test solutions, dissolve 0.10 g in 5 ml of water, add 0.5 ml of sodium hydroxide (~400 g/l) TS, 2 ml of ether R, and shake. Allow the layers to separate and use the upper layer as solution A. Dilute 0.5 ml of solution A to 50 ml with ether R and use as solution B. Apply separately to the plate 5 µl of each of solutions A and B. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes, return it to the chamber, and allow the solvent mixture to ascend a second time 12 cm above the line of application. Remove the plate, allow it to dry in air for 10 minutes, and spray it with dichlorofluorescein TS. Allow it to stand for 5 minutes and examine the chromatogram in daylight; red to orange spots on a white to almost white background are obtained. Then examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.20 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 28.38 mg of $C_{15}H_{21}NO_2$,HCl.

Additional requirements for Pethidine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

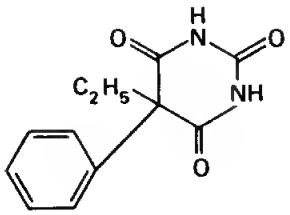
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.4 IU of endotoxin RS per mg.

PHENOBARBITALUM
PHENOBARBITAL

Molecular formula. $C_{12}H_{12}N_2O_3$

Relative molecular mass. 232.2

Graphic formula.



Chemical name. 5-Ethyl-5-phenylbarbituric acid; 5-ethyl-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione; CAS Reg. No. 50-06-6.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in about 1100 parts of water, in about 10 parts of ethanol (~750 g/l) TS and in about 15 parts of ether R.

Category. Hypnotic; sedative; anticonvulsant.

Storage. Phenobarbital should be kept in a well-closed container.

Additional information. Phenobarbital may exhibit polymorphism.

Requirements

Definition. Phenobarbital contains not less than 98.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of phenobarbital.

B. Dissolve 20 mg in 5 ml of ethanol (~750 g/l) TS, add 1 drop of cobaltous chloride TS and 1 drop of ammonia (~100 g/l) TS; a violet colour is produced.

C. Shake for 3 minutes 0.1 g with 4 ml of sodium hydroxide (0.1 mol/l) VS and 1 ml of water. Filter and to 2 ml of the filtrate add 4 drops of mercuric chloride (65 g/l) TS; a white precipitate is formed, which dissolves on the addition of 5 ml of ammonia (~100 g/l) TS.

D. Dissolve 0.1 g in 2 ml of sulfuric acid (~1760 g/l) TS, add about 10 mg of sodium nitrite R, and warm on a water-bath for 10 minutes; an orange-yellow colour with a brownish sheen is produced.

Melting range. 174–178 °C.

Solution in alkali. Dissolve 1.0 g in 4.0 ml of sodium hydroxide (~80 g/l) TS and add 6.0 ml of water; the solution is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Acidity. Boil 1.0 g with 50 ml of water for 2 minutes, adjust the volume to 50 ml, and filter. To 10 ml of the filtrate add 0.15 ml of methyl red/ethanol TS; not more than 0.1 ml of sodium hydroxide (0.1 mol/l) VS is required to obtain the midpoint of the indicator (orange).

Phenylbarbituric acid. Boil 1.0 g with 5 ml of ethanol (~750 g/l) TS for 3 minutes under a reflux condenser; a clear solution is produced.

Neutral and basic impurities. Dissolve 1.0 g in a mixture of 5 ml of sodium hydroxide (~80 g/l) TS and 10 ml of water and shake for 1 minute with 25 ml of ether R. Wash the ethereal layer 3 times, each time with 5 ml of water, evaporate the ether, and dry the residue at 105 °C for 1 hour; the residue weighs not more than 3.0 mg.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of chloroform R, 15 volumes of ethanol (~750 g/l) TS, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 10 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.20 g, accurately weighed, in 30 ml of dimethylformamide R, add 2 drops of thymolphthalein/dimethylformamide TS and titrate with sodium methoxide (0.1 mol/l) VS to a blue endpoint, as described under 2.6 Non-aqueous titration. Method B. Each ml of sodium methoxide (0.1 mol/l) VS is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

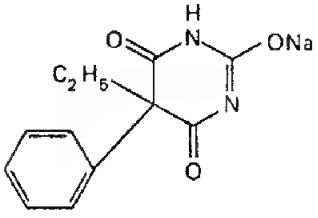
PHENOBARBITALUM NATRICUM

PHENOBARBITAL SODIUM

Molecular formula. $C_{12}H_{11}N_2NaO_3$

Relative molecular mass. 254.2

Graphic formula.



Chemical name. Sodium 5-ethyl-5-phenylbarbiturate; 5-ethyl-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione monosodium salt; CAS Reg. No. 57-30-7.

Description. White, crystalline granules or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Hypnotic; sedative; anticonvulsant.

Storage. Phenobarbital sodium should be kept in a tightly closed container, protected from light.

Additional information. Phenobarbital sodium is hygroscopic. Even in the absence of light, Phenobarbital sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Phenobarbital sodium contains not less than 98.0% and not more than 101.0% of $C_{12}H_{11}N_2NaO_3$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of phenobarbital sodium.

B. Dissolve 0.2 g in 10 ml of water and add 2 ml of hydrochloric acid (~70 g/l) TS; a white, crystalline precipitate is produced. Wash the precipitate with water until free from chlorides, and dry at 105 °C; melting temperature, about 175 °C (phenobarbital). Keep the precipitate for test C.

C. Dissolve 20 mg of the precipitate obtained from test B in 5 ml of ethanol (~750 g/l) TS, add 1 drop of cobaltous chloride TS and 1 drop of ammonia (~100 g/l) TS; a violet colour is produced.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R remains clear and colourless during 15 minutes.

Loss on drying. Dry to constant weight at 140 °C; it loses not more than 70 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 9.0–10.8.

Neutral and basic impurities. Dissolve 1.0 g in a mixture of 5 ml of sodium hydroxide (~80 g/l) TS and 10 ml of water, and shake for 1 minute with 25 ml of ether R. Wash the ethereal layer 3 times, each time with 5 ml of water, evaporate the ether, and dry the residue at 105 °C for 1 hour; the residue weighs not more than 3.0 mg.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of chloroform R, 15 volumes of ethanol (~750 g/l) TS and 5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 10 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

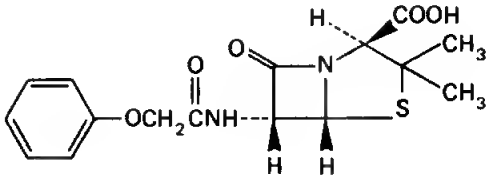
Assay. Dissolve about 0.5 g, accurately weighed, in 15 ml of water, add 5 ml of hydrochloric acid (2 mol/l) VS, and extract with 50 ml of ether R and then with successive quantities, each of 25 ml of ether R, until complete extraction has been effected. Wash the combined extracts twice with water, using 5 ml each time. Add the ether extract to the main ether extract, evaporate to low bulk, add 2 ml of dehydrated ethanol R, evaporate to dryness, and dry the residue to constant weight at 105 °C. Each g of residue is equivalent to 1.095 g of $C_{12}H_{11}N_2NaO_3$.

PHENOXYMETHYLPENICILLINUM
PHENOXYMETHYLPENICILLIN

Molecular formula. $C_{16}H_{18}N_2O_5S$

Relative molecular mass. 350.4

Graphic formula.



Chemical name. (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6 [(phenoxyacetyl)amino]-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid; CAS Reg. No. 87-08-1.

Description. A white, fine crystalline powder.

Solubility. Soluble in 1700 parts of water and in 7 parts of ethanol (~750 g/l) TS.

Category. Antibiotic.

Storage. Phenoxymethylpenicillin should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Phenoxymethylpenicillin is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Phenoxymethylpenicillin contains not less than 95.0% and not more than 102.0% of $C_{16}H_{18}N_2O_5S$, calculated with reference to the anydrous substance.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenoxymethylpenicillin RS or with the *reference spectrum* of phenoxymethylpenicillin.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless.

C. Place 2 mg in a test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is red. Immerse the test-tube for 1 minute in a water-bath; a red-brown colour is produced.

Specific optical rotation. Use a 10 mg/ml solution in 1-butanol R and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C}$ = +186 to +200°.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method B, using about 0.3 g of the substance; not more than 15 mg/g.

pH value. pH of a 5.0 mg/ml suspension in water, 2.4–4.0.

***p*-Hydroxyphenoxymethylpenicillin.** Dissolve about 0.1 g, accurately weighed, in sufficient sodium hydroxide (0.1 mol/l) VS to produce 100 ml.

Measure the absorbance of a 1-cm layer at the maximum at about 306 nm; not more than 0.36 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Ultraviolet absorbance range. Dilute 20 ml of the solution obtained in the test for *p*-hydroxyphenoxymethylpenicillin to 100 ml with sodium hydroxide (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer at the maximum at about 274 nm; not less than 0.56 and not more than 0.62.

Assay. Dissolve about 50 mg, accurately weighed, in a mixture of 0.6 ml of sodium hydrogen carbonate (40 g/l) TS and 10 ml of water and dilute with sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place it in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A). To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

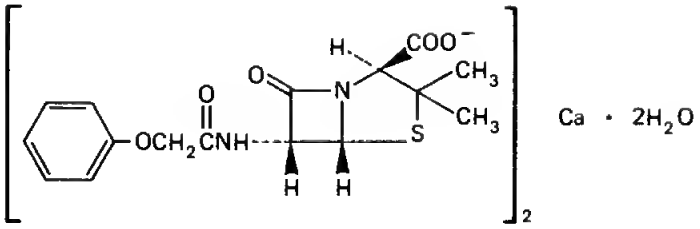
From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{18}N_2O_5S$ in the substance being examined by comparison with phenoxymethylpenicillin potassium RS similarly and concurrently examined, but omitting the addition of sodium hydrogen carbonate (40 g/l) TS; each mg of phenoxymethylpenicillin potassium RS ($C_{16}H_{17}KN_2O_5S$) is equivalent to 0.902 mg of phenoxymethylpenicillin ($C_{16}H_{18}N_2O_5S$). In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.63 ± 0.03.

PHENOXYMETHYLPENICILLINUM CALCICUM
PHENOXYMETHYLPENICILLIN CALCIUM

Molecular formula. $(C_{16}H_{17}N_2O_5S)_2Ca,2H_2O$ or $C_{32}H_{34}CaN_4O_{10}S_2,2H_2O$

Relative molecular mass. 774.9

Graphic formula.



Chemical name. Calcium bis[(2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] dihydrate; calcium bis[[2*S*-(2α,5α,6β)]3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] dihydrate; CAS Reg. No. 73368-74-8.

Description. A white, fine crystalline powder; odourless or with a faint characteristic odour.

Solubility. Slowly soluble in 120 parts of water.

Category. Antibiotic.

Storage. Phenoxymethylpenicillin calcium should be kept in a well-closed container.

Additional information. Even in the absence of light, Phenoxymethylpenicillin calcium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Phenoxymethylpenicillin calcium contains not less than 95.0% and not more than 102.0% of $(C_{16}H_{17}N_2O_5S)_2Ca$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenoxymethylpenicillin calcium RS or with the *reference spectrum* of phenoxymethylpenicillin calcium.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless.

C. Place 2 mg in a test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is red. Immerse the test-tube for 1 minute in a water-bath; a red-brown colour is produced.

D. Ignite a small quantity, dissolve the residue in hydrochloric acid (~70 g/l) TS, and make the solution alkaline by the addition of ammonia (~100 g/l) TS; the solution yields the reactions described under 2.1 General identification tests as characteristic of calcium.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; not more than 50 mg/g.

pH value. pH of a 5.0 mg/ml solution in carbon-dioxide-free water R, 5.0–7.5.

***p*-Hydroxyphenoxymethylpenicillin.** Dissolve about 0.11 g, accurately weighed, in sufficient sodium hydroxide (0.1 mol/l) VS to produce 100 ml. Measure the absorbance of a 1-cm layer at the maximum at about 306 nm; not more than 0.36 (use preferably 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Ultraviolet absorbance range. Dilute 20 ml of the solution obtained in the test for *p*-hydroxyphenoxymethylpenicillin to 100 ml with sodium hydroxide (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer at the maximum at about 274 nm; not less than 0.56 and not more than 0.62.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place it in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A). To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

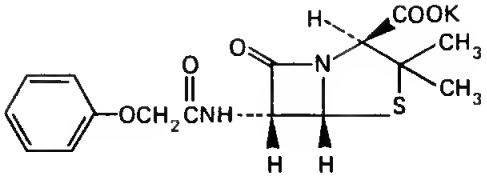
From the difference between the absorbance of solution A and that of solution B, calculate the amount of $(C_{16}H_{17}N_2O_5S)_2Ca$ in the substance being tested by comparison with phenoxymethylpenicillin potassium RS, similarly and concurrently examined, taking into account that each mg of phenoxymethylpenicillin potassium RS ($C_{16}H_{17}KN_2O_5S$) is equivalent to 0.951 mg of phenoxymethylpenicillin calcium $(C_{16}H_{17}N_2O_5S)_2Ca$. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.63 ± 0.03.

PHENOXYMETHYLPENICILLINUM KALICUM
PHENOXYMETHYLPENICILLIN POTASSIUM

Molecular formula. $C_{16}H_{17}KN_2O_5S$

Relative molecular mass. 388.5

Graphic formula.



Chemical name. Potassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate; potassium [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylate; CAS Reg. No. 132-98-9.

Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.

Solubility. Soluble in about 1.5 parts of water; practically insoluble in ether R.

Category. Antibiotic.

Storage. Phenoxymethylpenicillin potassium should be kept in a tightly closed container; protected from light.

Additional information. Even in the absence of light, Phenoxymethylpenicillin potassium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Phenoxymethylpenicillin potassium contains not less than 95.0% and not more than 102.0% of $C_{16}H_{17}KN_2O_5S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenoxymethylpenicillin potassium RS or with the *reference spectrum* of phenoxymethylpenicillin potassium.

B. To 2 mg in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless.

C. Place 2 mg in a test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is red. Immerse the test-tube for 1 minute in a water-bath; a red-brown colour is produced.

D. Ignite a small quantity, dissolve the residue in water and filter. To the filtrate, add 2 ml of sodium hydroxide (~80 g/l) TS; it yields the reaction described under 2.1 General identification tests as characteristic of potassium.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +215$ to $+235\,°$.

Clarity of solution. A solution of 0.2 g in 10 ml of water is not more than slightly opalescent.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

pH value. pH of a 5.0 mg/ml solution in carbon-dioxide-free water R, 5.0–7.5.

***p*-Hydroxyphenoxymethylpenicillin.** Dissolve about 0.11 g, accurately weighed, in sufficient sodium hydroxide (0.1 mol/l) VS to produce 100 ml. Measure the absorbance of a 1-cm layer at the maximum at about 306 nm; not more than 0.36 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Ultraviolet absorbance range. Dilute 20 ml of the solution obtained in the test for *p*-hydroxyphenoxymethylpenicillin to 100 ml with sodium hydroxide (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer at the maximum at about 274 nm; not less than 0.56 and not more than 0.62.

Assay. Dissolve about 50 mg, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place it in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A). To the second tube add 10.0 ml of Water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{17}KN_2O_5S$ in the substance being tested by comparison with phenoxymethylpenicillin potassium RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.63 ± 0.03.

PHENYLHYDRARGYRI NITRAS
PHENYLMERCURIC NITRATE

$C_{12}H_{11}Hg_2NO_4$

Chemical name. Nitratophenylmercury; (nitrato-*O*)phenylmercury; CAS Reg. No. 55-68-5.

Description. White, lustrous plates or a white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS; soluble in glycerol R and in fixed oils.

Category. Antimicrobial preservative.

Storage. Phenylmercuric nitrate should be kept in a tightly closed container, protected from light.

Additional information. Phenylmercuric nitrate is affected by light. It melts at about 188 °C with decomposition.

Requirements

Definition. Phenylmercuric nitrate is a mixture of phenylmercuric nitrate and phenylmercuric hydroxide.

Phenylmercuric nitrate contains not less than **98.0%** and not more than the equivalent of **101.0%** of $C_{12}H_{11}Hg_2NO_4$, calculated with reference to the dried substance.

Identity tests

A. To 10 ml of a saturated solution add 2 drops of sodium sulfide TS; a white precipitate is produced. Boil the mixture and allow to stand; the precipitate becomes black.

B. Heat 0.5 g with 0.5 g of zinc R powder, 0.5 g of reduced iron R, and 5 ml of sodium hydroxide (~200 g/l) TS. Place a piece of moistened red litmus paper R over the vapours; the colour of the paper changes to blue.

C. To 10 ml of a saturated solution add 1 ml of hydrochloric acid (~70 g/l) TS and heat to boiling; a white precipitate is produced. Filter, and to 5 ml of the filtrate add 2 ml of ferrous sulfate (15 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of nitrates.

Mercuric salts and heavy metals. Heat 0.1 g with 15 ml of water, cool, filter, and add 0.1 ml of sodium sulfide TS to the filtrate; a precipitate is produced, the colour of which remains unchanged.

Residue on ignition. Not more than 5.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 10 mg/g.

Acidity. To a 0.2 mg/ml solution add 3 drops of bromocresol green/ethanol TS; the solution is neutral (green).

Assay. Transfer about 0.2 g, accurately weighed, to a conical flask, and dissolve in 90 ml of water and 10 ml of nitric acid (~1000 g/l) TS. Add 2 ml of ferric ammonium sulfate (45 g/l) TS and titrate with ammonium thiocyanate (0.05 mol/l) VS.

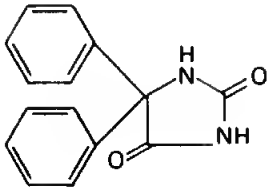
Each ml of ammonium thiocyanate (0.05 mol/l) VS is equivalent to 0.01586 g of $C_{12}H_{11}Hg_2NO_4$.

PHENYTOINUM
PHENYTOIN

Molecular formula. $C_{15}H_{12}N_2O_2$

Relative molecular mass. 252.3

Graphic formula.



Chemical name. 5,5-Diphenylhydantoin; 5,5-diphenyl-2,4-imidazolidinedione; CAS Reg. No. 57-41-0.

Description. A white, crystalline powder; odourless.

Solubility. Practically insoluble in water; sparingly soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Anticonvulsant.

Storage. Phenytoin should be kept in a tightly closed container, protected from light.

Requirements

Definition. Phenytoin contains not less than 98.0% and not more than 101.0% of $C_{15}H_{12}N_2O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenytoin RS or with the *reference spectrum* of phenytoin.

B. Dissolve 20 mg in 2 ml of ammonia (~100 g/l) TS and add 5 ml of silver nitrate (40 g/l) TS; a white precipitate is produced.

C. Dissolve 5 mg in 1 ml of boiling ethanol (~750 g/l) TS, add 2 ml of water, 2 drops of pyridine R, and 2 drops of copper (II) sulfate (80 g/l) TS; allow to cool; a blue-violet, crystalline precipitate is produced.

D. Melting temperature, about 295 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution, as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Acidity or alkalinity. Shake 2 g for 1 minute with 40 ml of carbon-dioxide-free water R, and filter. To 10 ml of the filtrate add 2 drops of phenolphthalein/ethanol TS; no colour is produced. Add 0.15 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS; a pink colour is produced. Add 0.3 ml of hydrochloric acid (0.01 mol/l) VS and 5 drops of methyl red/ethanol TS; a red or orange colour is produced.

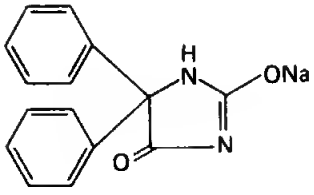
Assay. Dissolve about 0.5 g, accurately weighed, in 50 ml of dimethylformamide R, add 2 drops of thymol blue/dimethylformamide TS and titrate with sodium methoxide (0.1 mol/l) VS to a blue endpoint, as described under 2.6 Non-aqueous titration, Method B. Each ml of sodium methoxide (0.1 mol/l) VS is equivalent to 25.23 mg of $C_{15}H_{12}N_2O_2$.

PHENYTOINUM NATRICUM
PHENYTOIN SODIUM

Molecular formula. $C_{15}H_{11}N_2NaO_2$

Relative molecular mass. 274.3

Graphic formula.



Chemical name. 5,5-Diphenylhydantoin monosodium salt; 5,5-diphenyl-2,4-imidazolidinedione monosodium salt; CAS Reg. No. 630-93-3.

Description. A white powder; odourless.

Solubility. Soluble in water, giving a slightly turbid solution owing to partial hydrolysis; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Anticonvulsant.

Storage. Phenytoin sodium should be kept in a tightly closed container.

Additional information. Phenytoin sodium is somewhat hygroscopic and on exposure to air gradually absorbs carbon dioxide.

Requirements

Definition. Phenytoin sodium contains not less than 98.5% and not more than 101.0% of $C_{15}H_{11}N_2NaO_2$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, D, and E may be applied.

A. Shake 0.1 g with 20 ml of water, acidify with hydrochloric acid (~70 g/l) TS, and extract with chloroform R; wash the chloroform extract with water and evaporate to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenytoin RS or with the *reference spectrum* of phenytoin.

B. Dissolve 0.1 g in a mixture of 1 ml of pyridine R and 9 ml of water, add 1 ml of copper (II) sulfate/pyridine TS, and allow to stand for 10 minutes; a blue precipitate is produced.

C. Dissolve 10 mg in 1 ml of water, add 1 drop of ammonia (~100 g/l) TS and heat until boiling begins. Add 1 drop of copper (II) sulfate/ammonia TS and shake; a pink, crystalline precipitate is formed.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

E. Shake 0.1 g with 20 ml of water, acidify with hydrochloric acid (~70 g/l) TS, and extract with chloroform R; wash the chloroform extract with water and evaporate to dryness. Melting temperature of the residue, about 295 °C (phenytoin).

Heavy metals. To 1.0 g add 24 ml of water and 6 ml of hydrochloric acid (~70 g/l) TS; heat the mixture until boiling begins. Filter, cool, and filter again through a suitable sintered glass filter. Dilute to 40 ml with water, mix, and determine the content of heavy metals as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 10 µg/g.

Solution in alkali. To 20 mg add 8.0 ml of carbon-dioxide-free water R and then add gradually 2.0 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 30 mg/g.

Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 3 drops of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.43 mg of $C_{15}H_{11}N_2NaO_2$.

Additional requirements for Phenytoin sodium for parenteral use

Complies with the monograph for "Parenteral preparations".

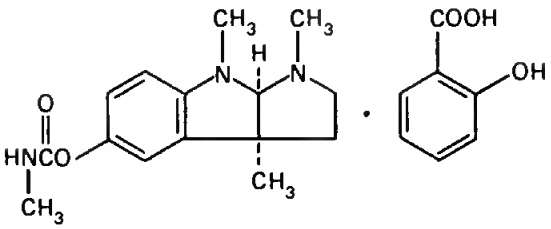
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.3 IU of endotoxin RS per mg.

PHYSOSTIGMINI SALICYLAS
PHYSOSTIGMINE SALICYLATE

Molecular formula. $C_{15}H_{21}N_3O_2,C_7H_6O_3$ or $C_{22}H_{27}N_3O_5$

Relative molecular mass. 413.5

Graphic formula.



Chemical name. Physostigmine monosalicylate; (3a*S*-*cis*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-ol, methylcarbamate (ester), mono-(2-hydroxybenzoate); CAS Reg. No. 57-64-7.

Other name. Eserine salicylate.

Description. Colourless crystals; odourless.

Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Anticholinesterase; miotic.

Storage. Physostigmine salicylate should be kept in a tightly closed container, protected from light, and preferably in quantities not exceeding 1 g.

Additional information. Physostigmine salicylate is very poisonous. It acquires a red tint when exposed to air or light. All tests should be performed on freshly prepared solutions.

Requirements

Definition. Physostigmine salicylate contains not less than 98.0% and not more than 101.0% of $C_{15}H_{21}N_3O_2,C_7H_6O_3$, calculated with reference to the dried substance.

Identity tests

A. To a 10 mg/ml solution add a few drops of sodium hydroxide (~80 g/l) TS; a white precipitate is produced, which gradually turns pink. It dissolves in an excess of the reagent to give a red solution.

B. Warm 10 mg with a few drops of ammonia (~100 g/l) TS; an orange solution is produced. Evaporate this solution and dissolve the residue in ethanol (~750 g/l) TS. To the resulting blue solution add a few drops of acetic acid (~300 g/l) TS; the colour is intensified. Dilute with water; a red fluorescence appears.

C. To a 10 mg/ml solution add a few drops of ferric chloride (25 g/l) TS; a violet colour, which remains after the addition of ethanol (~750 g/l) TS, appears.

D. Melting temperature, about 186 °C.

Specific optical rotation. Use a 10 mg/ml solution; $[\alpha]_D^{20\,°C} = -90$ to -94 °.

Clarity and colour of solution. A solution of 0.10 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution, 4.6–5.2.

Eseridine. To 5 ml of a 10 mg/ml solution add 5 drops of hydrochloric acid (~70 g/l) TS, 1.5 ml of potassium iodate (0.01 mol/l) VS and 1 ml of chloroform R. Shake for 1 minute; no violet colour develops in the chloroform layer.

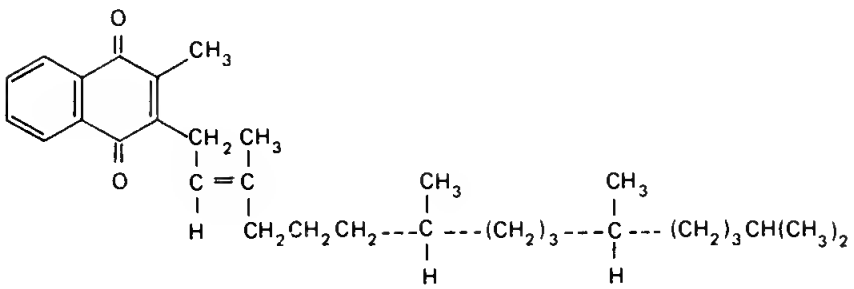
Assay. Dissolve about 0.35 g, accurately weighed, in 30 ml of a mixture of equal volumes of chloroform R and glacial acetic acid R1. Titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 41.35 mg of $C_{15}H_{21}N_3O_2,C_7H_6O_3$.

PHYTOMENADIONUM
PHYTOMENADIONE

Molecular formula. $C_{31}H_{46}O_2$

Relative molecular mass. 450.7

Graphic formula.



Chemical name. Phylloquinone; [*R*-[*R**,*R**-(*E*)]]-2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-1,4-naphthalenedione; 2-methyl-3-phytyl-1,4-naphthoquinone; CAS Reg. No. 84-80-0.

Other names. Phytonadione; Vitamin K_1.

Description. A clear, yellow to amber-coloured, very viscous liquid; odourless or almost odourless.

Miscibility. Practically immiscible with water; sparingly miscible with ethanol (~750 g/l) TS; freely miscible with ether R.

Category. Anticoagulant.

Storage. Phytomenadione should be kept in a tightly closed container, protected from light.

Requirements

Definition. Phytomenadione contains not less than 97.0% and not more than 102.0% of $C_{31}H_{46}O_2$.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in 2,2,4-trimethylpentane R, when observed between 230 nm and 350 nm, exhibits 4 maxima at about 243 nm, 249 nm, 261 nm, and 270 nm. The absorbances at those wavelengths using 1-cm cells are about 0.40, 0.42, 0.38, and 0.39, respectively. The spectrum also exhibits minima at about 246 nm, 254 nm, and 266 nm. The ratio of the absorbance at the minimum of about 254 nm to that at the maximum of about 249 nm is between 0.70 and 0.75.

B. The absorption spectrum of a 0.10 mg/ml solution in 2,2,4-trimethylpentane R, when observed between 230 nm and 350 nm, exhibits a maximum at about 327 nm and a minimum at about 285 nm. The absorbance of a 1-cm layer at the maximum is about 0.70, and at the minimum about 0.22.

C. Mix about 0.05 g of the test liquid with 5 ml of ethanol (~750 g/l) TS and add 1.0 ml of potassium hydroxide/ethanol TS1; a green colour is produced. Allow to stand for 15 minutes; the colour of the solution turns to red-brown.

Refractive index. $n_D^{20} = 1.525 - 1.529$.

Acidity or alkalinity. Dissolve 1 g in 20 ml of dehydrated ethanol R; the solution is neutral to litmus paper R.

Related substances. Carry out, in subdued light, the test described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 80 volumes of cyclohexane R, 20 volumes of ether R, and 1 volume of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in 2,2,4-trimethylpentane R containing (A) 5 mg of the test substance per ml and (B) 0.05 mg of menadione R per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

Carry out the following operations in subdued light.

Dissolve about 0.1 g, accurately weighed, in sufficient 2,2,4-trimethylpentane R to produce 100 ml. Dilute 10 ml to 100 ml with 2,2,4-trimethylpentane R and further dilute 10 ml of this solution to 100 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 249 nm and calculate the content of $C_{31}H_{46}O_2$, using the absorptivity value of 42 ($A^{1\%}_{1\,cm} = 420$).

Additional requirements for Phytomenadione for parenteral use

Complies with the monograph for "Parenteral preparations".

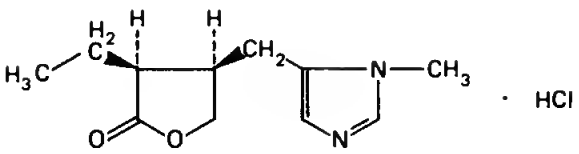
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 14.0 IU of endotoxin RS per mg.

PILOCARPINI HYDROCHLORIDUM
PILOCARPINE HYDROCHLORIDE

Molecular formula. $C_{11}H_{16}N_2O_2,HCl$

Relative molecular mass. 244.7

Graphic formula.



Chemical name. Pilocarpine monohydrochloride; (3*S*-*cis*)-3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2(3*H*)-furanone monohydrochloride; CAS Reg. No. 54-71-7.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; insoluble in ether R.

Category. Parasympathomimetic; miotic.

Storage. Pilocarpine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Pilocarpine hydrochloride is very poisonous; it is hygroscopic and is affected by light. Even in the absence of light, Pilocarpine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Pilocarpine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{11}H_{16}N_2O_2$,HCl, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 5 ml of water, add 2 drops of sulfuric acid (~100 g/l) TS, 1 ml of hydrogen peroxide (~60 g/l) TS, 1 ml of toluene R, and 1 drop of potassium dichromate (100 g/l) TS, and shake well; the toluene layer acquires a violet colour, whereas the aqueous layer remains yellow.

B. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

C. Melting temperature, about 203 °C.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = +89 to +93 °.

Nitrates. Dissolve 0.05 g in 5 ml of water and carefully add the solution to 5 ml of a 1 mg/ml solution of diphenylamine R in sulfuric acid (~1760 g/l) TS, ensuring that the liquids do not mix; no blue colour is produced at the interface of the two liquids.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 3.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

pH value. pH of a 5.0 mg/ml solution, 3.8–5.2.

Related alkaloids. Carry out the tests as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 25 volumes of chloroform R, 20 volumes of acetone R, and 0.4 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 50 mg of the test substance per ml and (B)

1.0 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.47 mg of $C_{11}H_{16}N_2O_2,HCl$.

Additional requirement for Pilocarpine hydrochloride for sterile use

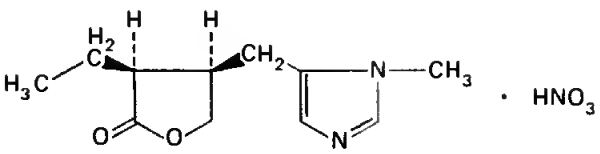
Complies with 3.2.1 Test for sterility of non-injectable preparations.

PILOCARPINI NITRAS
PILOCARPINE NITRATE

Molecular formula. $C_{11}H_{16}N_2O_2,HNO_3$

Relative molecular mass. 271.3

Graphic formula.



Chemical name. Pilocarpine mononitrate; (3*S*-*cis*)-3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]-2(3*H*)-furanone mononitrate; CAS Reg. No. 148-72-1.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Parasympathomimetic; miotic.

Storage. Pilocarpine nitrate should be kept in a tightly closed container, protected from light.

Additional information. Pilocarpine nitrate is very poisonous; it is affected by light. Even in the absence of light, Pilocarpine nitrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Pilocarpine nitrate contains not less than 98.5% and not more than 101.0% of $C_{11}H_{16}N_2O_2,HNO_3$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 5 ml of water, add 2 drops of sulfuric acid (~100 g/l) TS, 1 ml of hydrogen peroxide (~60 g/l) TS, 1 ml of toluene R, and 1 drop of potassium dichromate (100 g/l) TS, and shake well; the toluene layer acquires a violet colour, whereas the aqueous layer remains yellow.

B. To 2 ml of a 0.05 g/ml solution add 2 ml of ferrous sulfate (15 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of nitrates.

C. Melting temperature, about 176 °C with decomposition.

Specific optical rotation. Use a 50 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +80$ to $+83$ °.

Chlorides. Dissolve 0.7 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.35 mg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

pH value. pH of a 5.0 mg/ml solution in water, 3.5–4.5.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 25 volumes of chloroform R, 20 volumes of acetone R, and 0.4 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 50 mg of the test substance per ml and (B) 1.0 mg of the test substance per ml. After removing the plate from the chro-

matographic chamber, allow it to dry in air, spray with potassium iodobismuthate TS2, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.13 mg of $C_{11}H_{16}N_2O_2,HNO_3$.

Additional requirement for Pilocarpine nitrate for sterile use

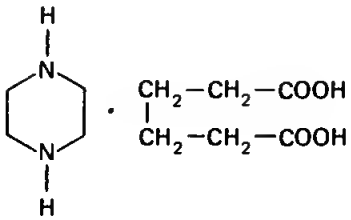
Complies with 3.2.1 Test for sterility of non-injectable preparations.

PIPERAZINI ADIPAS
PIPERAZINE ADIPATE

Molecular formula. $C_4H_{10}N_2,C_6H_{10}O_4$ or $C_{10}H_{20}N_2O_4$

Relative molecular mass. 232.3

Graphic formula.



Chemical name. Piperazine hexanedioate (1:1); hexahydro-1,4-diazine adipate (1:1); CAS Reg. No. 142-88-1.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Anthelmintic.

Storage. Piperazine adipate should be kept in a well-closed container.

Requirements

Definition. Piperazine adipate contains not less than 98.0% and not more than 101.0% of $C_4H_{10}N_2,C_6H_{10}O_4$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.1 g in 5 ml of water, add 0.5 g of sodium hydrogen carbonate R, 0.5 ml of freshly prepared potassium ferricyanide (50 g/l) TS, and 0.1 ml of mercury R. Shake vigorously for 1 minute, and allow to stand for 20 minutes; a reddish colour slowly develops.

B. Dissolve 0.5 g in 10 ml of water and add 5 ml of hydrochloric acid (~250 g/l) TS. Extract 3 times with ether R, using 10 ml each time, and keep the aqueous layer for test C. Evaporate the ether extracts to dryness and dry at 105 °C: melting temperature, about 152 °C (adipic acid).

C. Cautiously heat the aqueous layer obtained from test B to eliminate any dissolved ether. Cool and add 0.5 g of sodium nitrite R. Heat to boiling and cool in ice for 15 minutes, stirring if necessary to induce crystallization. Filter, wash with 10 ml of ice-water and dry the precipitate at 105 °C; melting temperature, about 158 °C (*N,N'*-dinitrosopiperazine).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.05 g/ml solution, 5.0–6.0.

Primary amines. For the preparation of the test solution dissolve 0.25 g in sufficient water to produce 50 ml. Transfer 0.5 ml of this solution to a test-tube. Separately transfer to a second test-tube 0.5 ml of a solution containing 10 µg/ml of ethylenediamine R to serve as a reference solution. To both tubes add 0.5 ml of ethanol (~750 g/l) TS, 1 ml of diethoxytetrahydrofuran/acetic acid TS, heat on a water-bath at 80 °C for 30 minutes, cool in ice, and add 3 ml of 4-dimethylaminobenzaldehyde TS4. Measure the absorbance at about 570 nm, 7–10 minutes after the addition of the last reagent, against a solvent cell containing the reagents prepared in a similar manner. The absorbance of the test solution is not more intense than that of the reference solution.

Assay. Dissolve about 0.20 g, accurately weighed, in 3.5 ml of sulfuric acid (0.5 mol/l) VS and 10 ml of water; add 100 ml of trinitrophenol (7 g/l) TS, heat

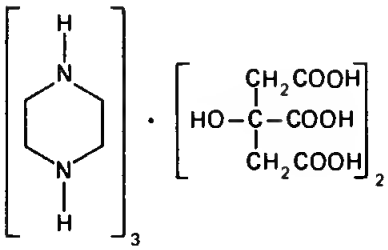
on a water-bath for 15 minutes, and allow to stand for 1 hour. Filter, wash the residue with successive quantities of trinitrophenol (7 g/l) TS, using 10 ml each time, until the washings are free from sulfates. Finally, wash with dehydrated ethanol R, and dry the residue to constant weight at 105 °C. Each g of residue is equivalent to 426.8 mg of $C_4H_{10}N_2,C_6H_{10}O_4$.

PIPERAZINI CITRAS
PIPERAZINE CITRATE

Molecular formula. $(C_4H_{10}N_2)_3,2C_6H_8O_7$ or $C_{24}H_{46}N_6O_{14}$ (anhydrous)

Relative molecular mass. 642.7 (anhydrous)

Graphic formula.



Chemical name. Piperazine 2-hydroxy-1,2,3-propanetricarboxylate (3:2); hexahydro-1,4-diazine citrate (3:2); CAS Reg. No. 144-29-6 (anhydrous).

Description. A fine, white, granular powder; almost odourless.

Solubility. Soluble in 1.5 parts of water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Anthelmintic.

Storage. Piperazine citrate should be kept in a well-closed container, protected from light.

Additional information. Piperazine citrate contains a variable amount of water of crystallization.

Requirements

Definition. Piperazine citrate contains not less than 98.0% and not more than 101.0% of $(C_4H_{10}N_2)_3,2C_6H_8O_7$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 0.1 g in 5 ml of water, add 0.5 g of sodium hydrogen carbonate R, 0.5 ml of freshly prepared potassium ferricyanide (50 g/l) TS, and 0.1 ml of mercury R. Shake vigorously for 1 minute, and allow to stand for 20 minutes; a reddish colour slowly develops.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of citrates.

C. Melting temperature, after drying at 105 °C, about 185 °C.

D. Dissolve 0.2 g in 5 ml of hydrochloric acid (~70 g/l) TS, and add 0.5 g of sodium nitrite R. Cool in ice for 15 minutes, stir if necessary to induce crystallization, filter, wash with 10 ml of ice-water, and dry the precipitate at 105 °C; melting temperature, about 158 °C (*N,N'*-dinitrosopiperazine).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 0.10 g/g and not more than 0.14 g/g.

pH value. pH of a 0.05 g/ml solution, 5.0–6.0.

Primary amines. For the preparation of the test solution dissolve 0.25 g in sufficient water to produce 50 ml. Transfer 0.5 ml of this solution to a test-tube. Separately transfer to a second test-tube 0.5 ml of a solution containing 10 µg/ml of ethylenediamine R to serve as a reference solution. To both tubes add 0.5 ml of ethanol (~750 g/l) TS, 1 ml of diethoxytetrahydrofuran/acetic acid TS, heat on a water-bath at 80 °C for 30 minutes, cool in ice, and add 3 ml of 4-dimethylaminobenzaldehyde TS4. Measure the absorbance at about 570 nm, 7–10 minutes after the addition of the last reagent, against a solvent cell containing the reagents prepared in a similar manner. The absorbance of the test solution is not more intense than that of the reference solution.

Assay. Dissolve about 0.20 g, accurately weighed, in 3.5 ml of sulfuric acid (0.5 mol/l) VS and 10 ml of water, add 100 ml of trinitrophenol (7 g/l) TS, heat on a water-bath for 15 minutes, and allow to stand for 1 hour. Filter, wash the residue with successive quantities of trinitrophenol (7 g/l) TS, using 10 ml each time, until the washings are free from sulfates. Finally, wash with dehydrated ethanol R, and dry the residue to constant weight at 105 °C. Each g of residue is equivalent to 393.5 mg of $(C_4H_{10}N_2)_3,2C_6H_8O_7$.

PIX LITHANTHRACIS
COAL TAR

Chemical name. Coal tar; CAS Reg. No. 8007-45-2.

Description. Brown-black or black, viscous liquid; odour, characteristic and strong resembling naphthalene.

Solubility. Slightly soluble in water; very slightly or partially soluble in ethanol (~750 g/l) TS, ether R and hexane R.

Category. Keratoplastic agent.

Storage. Coal tar should be kept in a tightly closed container.

Additional information. Coal tar bums in air with a luminous sooty flame. On exposure to air it hardens. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Coal tar is a by-product usually obtained during the destructive distillation of coal. It is a complex and undefined mixture of a great number of chemical compounds. The product is available in various compositions.

Identity test
Carefully add 0.5 g to 10 ml of light petroleum R1 and allow to stand for 30 minutes; the supernatant liquid has a blue fluorescence in daylight and a more intense fluorescence when viewed in ultraviolet light (365 nm).

Sulfated ash. Not more than 20 mg/g.

PODOPHYLLI RESINA
PODOPHYLLUM RESIN

Chemical name. Podophyllum resin; CAS Reg. No. 8050-60-0.

Other name. Podophyllinum.

Description. Light brown to greenish yellow or brownish grey masses or an amorphous powder.

Solubility. Practically insoluble in cold water; partially soluble in hot water, and ether R; soluble in ethanol (~750 g/l) TS.

Category. Keratolytic agent.

Storage. Podophyllum resin should be kept in a tightly closed container, protected from light, and preferably stored at a temperature between 2 and 15 °C.

Labelling. The designation on the container should state the botanical source.

Additional information. *CAUTION:* Podophyllum resin must be handled with care, avoiding contact with the skin and mucous membranes and inhalation of airborne particles.

On exposure to light or to temperatures above 25 °C, it becomes darker in colour.

Requirements

Definition. Podophyllum resin is a mixture of resins obtained from the rhizomes and roots of *Podophyllum hexandrum* Royle (*P. emodi* Wall.) or *Podophyllum peltatum* L. after percolation with ethanol and precipitation from water or very dilute acids.

Podophyllum resin contains not less than **40.0%** and not more than the equivalent of **52.5%** of Podophyllum toxin (α and β peltatum), calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 2 ml of ethanol (~750 g/l) TS and add 1 drop of ferric chloride (25 g/l) TS; a deep, dark green colour is produced and the solution appears black in reflected light.

B. Add 0.4 g, finely powdered, to 3 ml of ethanol (~535 g/l) TS, then add 0.5 ml of potassium hydroxide (1 mol/l) VS, shake gently, and allow to stand; the resin of *P. hexandrum* produces a stiff jelly, whereas the resin of *P. peltatum* does not gelatinize.

C. To two separate tubes add a few mg of Podophyllum resin, add potassium hydroxide (1 mol/l) VS to one tube and sodium hydroxide (1 mol/l) VS to the other, and allow to dissolve; a yellow solution is produced in both tubes which becomes darker on standing. Add a few drops of hydrochloric acid (~250 g/l) TS; a resin precipitates.

Matter insoluble in ethanol. Shake 1 g, finely powdered, with 20 ml of ethanol (~750 g/l) TS for 5 minutes. Filter through a sintered-glass crucible

(approx. porosity 40 µm), wash the filter with ethanol (~750 g/l) TS, and dry at 105 °C; the residue weighs not more than 25 mg.

Matter insoluble in ammonia. Shake 0.5 g, finely powdered, with 30 ml of ammonia (~100 g/l) TS for 30 minutes at about 20 °C. Filter through a sintered-glass crucible (approx. porosity 40 µm) and wash the flask and filter with 30 ml of water, the time taken for filtering and washing being not more than 10 minutes. Dry the filter with the residue to constant mass at 105 °C; the residue from the resin of *P. hexandrum* weighs not less than 0.18 g and not more than 0.30 g, whereas the residue from the resin of *P. peltatum* weighs not more than 50 mg.

Sulfated ash. Not more than 15 mg/g.

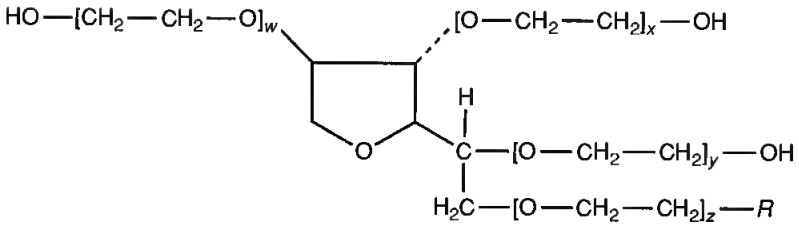
Loss on drying. Dry to constant mass at 105 °C; it loses not more than 50 mg/g.

Assay. Transfer about 0.45 g, finely powdered and accurately weighed, to a glass-stoppered 50-ml flask, add 15 ml of chloroform R, and shake for 30 minutes. Filter, then transfer 10 ml of the filtrate to a tared 100-ml flask, previously dried to constant mass at 70 °C and containing 80 ml of light petroleum R. Filter the precipitate through a tared sintered-glass crucible (approx. porosity 40 µm), previously dried to constant mass at 70 °C, and wash the flask and the filter with 20 ml of light petroleum R. Dry both of them at 70 °C for 1 hour, cool, and weigh. Determine the mass of the residue, taking into account the tare of the filter and the flask.

Each g of residue is equivalent to 0.30 g of Podophyllum resin.

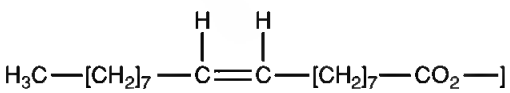
POLYSORBATA 20, 60, 80

POLYSORBATES 20, 60, 80



[Sum of *w*, *x*, *y* and *z* is 20;

R is $H_3C—[CH_2]_{10}—CO_2—$, $H_3C—[CH_2]_{16}—CO_2—$, or



Chemical names. Polysorbate 20: Polyoxyethylene 20 sorbitan monolaurate; sorbitan monododecanoate, poly(oxy-1,2-ethanediyl) derivatives; CAS Reg. No. 9005-64-5.

Polysorbate 60: Polyoxyethylene 20 sorbitan monostearate; sorbitan monooctadecanoate, poly(oxy-1,2-ethanediyl) derivatives; CAS Reg. No. 9005-67-8.

Polysorbate 80: Polyoxyethylene 20 sorbitan monooleate; sorbitan mono[(*Z*)-9-octadecenoate], poly(oxy-1,2-ethanediyl) derivatives; CAS Reg. No. 9005-65-6.

Description. Polysorbates 20 and 80 are yellowish or yellowish brown, oily liquids. Polysorbate 60 is a gelatinous mass, and a clear liquid above 25 °C.

Solubility. Miscible with water, ethanol (~750 g/l) TS, methanol R, and ethyl acetate R; practically insoluble in fatty oils and in liquid paraffin R.

Category. Nonionic surfactant.

Storage. Polysorbates should be kept in tightly closed containers, protected from light.

Additional information. Relative densities of Polysorbates 20 and 60 d_{20}^{20} = about 1.10; Polysorbate 80 d_{20}^{20} = about 1.08.

Requirements

Definition. Polysorbates are mixtures of partial fatty acid esters of sorbitol and its anhydrides, copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydride.

In Polysorbate 20 the fatty acid is lauric acid which may contain other fatty acids. In Polysorbate 60 the fatty acid is stearic acid which may contain other fatty acids, especially palmitic acid. In Polysorbate 80 the fatty acid is oleic acid.

Identity tests

A. A mixture of 6 volumes of Polysorbates and 4 volumes of water yields a gelatinous mass at room temperature as well as at lower temperatures.

B. Dissolve 0.1 g in 5 ml of chloroform R, and add 0.1 g of potassium thiocyanate R and 0.1 g of cobalt(II) nitrate R. Stir with a glass rod; the solution becomes blue.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Acid value. Not more than 2.0.

Hydroxyl value. Use Method B.

Polysorbate 20: 96–108.

Polysorbate 60: 81–96.

Polysorbate 80: 65–80.

Iodine value.

Polysorbates 20 and 60: not more than 5.0.

Polysorbate 80: 18–24.

Saponification value.

Polysorbate 20: 40–50.

Polysorbate 60: 45–55.

Polysorbate 80: 45–55.

Reducing impurities. Dissolve 2 g in 25 ml of hot water and add 25 ml of sulfuric acid (~100 g/l) TS and 0.1 ml of ferroin TS. Titrate with ceric ammonium nitrate (0.01 mol/l) VS, shaking continuously, until the colour change from red to greenish blue persists for 30 seconds. Repeat the procedure without the Polysorbates being examined and make any necessary corrections. Consumption of ceric ammonium nitrate (0.01 mol/l) VS:

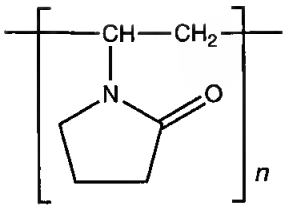
Polysorbates 20 and 60: not more than 2.0 ml

Polysorbate 80: not more than 5.0 ml.

Sulfated ash. Use 2.0 g; not more than 2.5 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 1 g; the water content is not more than 30 mg/g.

POVIDONUM
POVIDONE



$(C_6H_9NO)n$

Chemical name. 1-Vinyl-2-pyrrolidinone polymer; 1-ethenyl-2-pyrrolidinone homopolymer; CAS Reg. No. 9003-39-8.

Other name. Povidone, polyvinylpyrrolidone.

Description. A white to creamy white powder; odourless or almost odourless.

Solubility. Soluble in water, and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Tablet binder; tablet coating; viscosity-increasing agent.

Storage. Povidone should be kept in a tightly closed container.

Labelling. The designation on the container of Povidone should state its viscosity.

Additional information. Povidone is hygroscopic. It is not necessarily suitable for use as a plasma extender.

Requirements

Definition. Povidone consists of linear polymers of 1-vinyl-2-pyrrolidinone groups, the degree of polymerization of which results in polymers of various molecular masses ranging from about 10 000 to 700 000.

Povidone contains not less than **11.5%** and not more than the equivalent of **12.8%** of nitrogen N, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 0.5 g in 5 ml of water and add 10 ml of hydrochloric acid (1 mol/l) VS and 2 ml of potassium dichromate (100 g/l) TS; an orange-yellow precipitate is formed.

B. Dissolve 0.1 g in 1 ml of water and add 0.2 ml of 4-dimethylaminobenzaldehyde TS6 and 0.1 ml of sulfuric acid (~1760 g/l) TS; a pink colour is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g; the water content is not more than 50 mg/g.

Aldehydes. To 10 g add 180 ml of sulfuric acid (~440 g/l) TS and boil under a reflux condenser for 45 minutes. Cool, reassemble the apparatus, distil, and collect 100 ml of distillate in a flask that is placed in an ice-bath and contains 20 ml of hydroxylamine hydrochloride (70 g/l) TS previously adjusted to pH 3.1. Titrate the distillate with sodium hydroxide (0.1 mol/l) VS to pH 3.1. Repeat the procedure without the Povidone being examined and make any necessary corrections. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 4.405 mg of aldehyde, calculated as acetaldehyde. Not more than 4.6 ml of sodium hydroxide (0.1 mol/l) VS is required (2.0 mg/g).

Vinylpyrrolidinone. Dissolve 10 g in 80 ml of water, add 1 g of sodium acetate R and titrate with iodine (0.05 mol/l) VS until a permanent colour is obtained. Add an excess of 3.0 ml of iodine (0.05 mol/l) VS, allow to stand for 10 minutes, and titrate the excess iodine with sodium thiosulfate (0.1 mol/l) VS, adding starch TS towards the end of the titration. Repeat the procedure without the Povidone being examined and make any necessary corrections. Not more than 3.6 ml of iodine (0.05 mol/l) VS is required (2.0 mg/g).

Assay. Carry out Method A as described under 2.10 Determination of nitrogen, using about 0.3 g, accurately weighed, and 11 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Carefully add 1 ml of hydrogen peroxide (~330 g/l) TS down the wall of the flask, and heat. Repeat this addition three to six times, and continue to heat until a slightly green and clear solution is produced. Proceed with the distillation as described. Repeat the procedure without the Povidone being examined and make any necessary corrections.

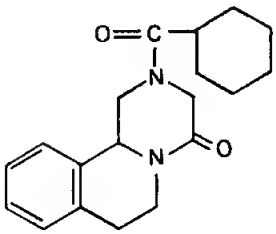
Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of N.

PRAZIQUANTELUM
PRAZIQUANTEL

Molecular formula. $C_{19}H_{24}N_2O_2$

Relative molecular mass. 312.4

Graphic formula.



Chemical name. 2-(Cyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4*H*-pyrazino-[2,1-α]isoquinolin-4-one; CAS Reg. No. 55268-74-1.

Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.

Solubility. Very slightly soluble in water; freely soluble in ethanol (~750 g/l) TS.

Category. Antischistosomal drug.

Storage. Praziquantel should be kept in a well-closed container, protected from light.

Requirements

Definition. Praziquantel contains not less than 98.5% and not more than 101.0% of $C_{19}H_{24}N_2O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from praziquantel RS or with the *reference spectrum* of praziquantel.

B. See the test below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Melting temperature, about 138 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 50 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 2 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 85 volumes of toluene R and 15 volumes of methanol R as the mobile phase. Use an unlined chamber. Apply separately to the plate, in a current of nitrogen R, 10 µl of each of 2 solutions in chloroform R containing (A) 50 mg of the test substance per ml and (B) 50 mg of praziquantel RS per ml; further apply 2 µl of each of 2 solutions in chloroform R containing (C) 0.5 mg of praziquantel RS per ml and (D) 1.0 mg of praziquantel RS per ml. Allow the mobile phase to ascend 7 cm. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, place the plate in a chamber with iodine vapours and allow to stand for 20 minutes. Examine the chromatogram immediately in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C, except one spot above the main spot which is not more intense than that obtained with solution D.

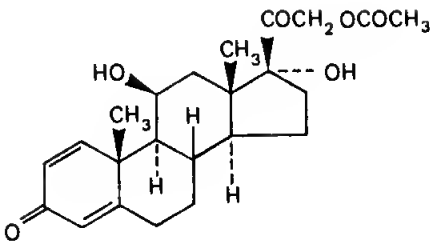
Assay. Dissolve about 0.04 g, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml. Measure the absorbance of a 1-cm layer at the maximum at about 265 nm. Calculate the amount of $C_{19}H_{24}N_2O_2$ in the substance being tested by comparison with praziquantel RS, similarly and concurrently examined.

PREDNISOLONI ACETAS
PREDNISOLONE ACETATE

Molecular formula. $C_{23}H_{30}O_6$

Relative molecular mass. 402.5

Graphic formula.



Chemical name. 11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-acetate; 21-(acetyloxy)-11β,17-dihydroxypregna-1,4-diene-3,20-dione; CAS Reg. No. 52-21-1.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS, and acetone R.

Category. Adrenal hormone.

Storage. Prednisolone acetate should be kept in a well-closed container, protected from light.

Additional information. Prednisolone acetate has a melting temperature of about 235 °C with decomposition.

Requirements

Definition. Prednisolone acetate contains not less than 96.0% and not more than 104.0% of $C_{23}H_{30}O_6$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone acetate RS or with the *reference spectrum* of prednisolone acetate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and

allow it to stand at room temperature until the solvents have completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use 75 volumes of toluene R and 25 volumes of chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of prednisolone acetate RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat it at 120 °C for 15 minutes, spray it with a mixture of 20 ml of sulfuric acid (~190 g/l) TS and 80 ml of ethanol (~750 g/l) TS, and then heat it at 120 °C for 10 minutes. Allow it to cool and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To 0.05 g add 2 ml of ethanol (~750 g/l) TS and 2 ml of sulfuric acid (~700 g/l) TS and boil gently for 1 minute; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

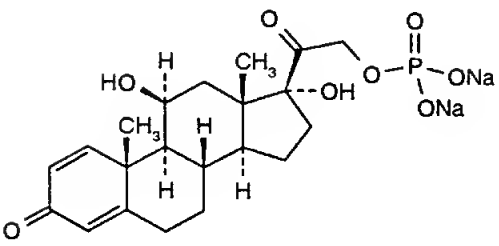
Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +112$ to + 119 °.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 95 volumes of dichloroethane R, 5 volumes of methanol R, and 0.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, then heat it at 105 °C for 10 minutes. Allow it to cool, spray it with blue tetrazolium/sodium hydroxide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 243 nm. Calculate the amount of $C_{23}H_{30}O_6$ in the substance being tested by comparison with prednisolone acetate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.37 ± 0.02.

PREDNISOLONI NATRII PHOSPHAS
PREDNISOLONE SODIUM PHOSPHATE



$C_{21}H_{27}Na_2O_8P$

Relative molecular mass. 484.4

Chemical name. 11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(disodium phosphate); (11β) 11,17-dihydroxy-21-(phosphonooxy)-pregna-1,4-diene-3,20-dione disodium salt; CAS Reg. No. 125-02-0.

Description. A white to light yellow, crystalline powder or granules.

Solubility. Freely soluble in water; soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS and acetone R.

Category. Corticosteroid.

Storage. Prednisolone sodium phosphate should be kept in a tightly closed container, protected from light.

Labelling. The designation Prednisolone sodium phosphate for sterile noninjectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

Additional information. Prednisolone sodium phosphate is hygroscopic.

Requirements

Prednisolone sodium phosphate contains not less than **96.0%** and not more than **103.0%** of $C_{21}H_{27}Na_2O_8P$, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A, D, and E or tests B, C, D, and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone sodium phosphate RS or with the *reference spectrum* of prednisolone sodium phosphate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a freshly prepared mixture of 3 volumes of 1-butanol R, 1 volume of acetic anhydride R, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of 4 solutions in methanol R containing (A) 2.5 mg of Prednisolone sodium phosphate per ml, (B) 2.5 mg of prednisolone sodium phosphate RS per ml, (C) a mixture of equal volumes of solutions A and B, and (D) equal volumes of solution A and a solution of 2.5 mg of dexamethasone sodium phosphate RS per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray with a mixture of 10 ml of sulfuric acid (~1760 g/l) TS and 90 ml of ethanol (~750 g/l) TS, heat at 120 °C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (365 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The principal spot obtained with solution C appears as a single compact spot, whereas the chromatogram obtained with solution D shows two spots which may not be completely separated.

C. To about 2 mg add 2 ml of sulfuric acid (~1760 g/l) TS and shake to dissolve; an intense red colour is produced within 5 minutes. Examine under ultraviolet light (365 nm); a reddish brown fluorescence is observed. Add the solution to 10 ml of water and mix; the colour fades and a greenish yellow fluorescence is produced.

D. When tested for sodium as described under 2.1 General identification tests, it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

E. To 1 ml of a 20 mg/ml solution add 3 ml of nitric acid (~130 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +95°$ to $+102°$.

Clarity and colour of solution. A solution of 0.5 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour Bn1 when compared as described under 1.11 Colour of liquids.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; not more than 0.080 g/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 7.5–9.0.

Inorganic phosphates. Dissolve 0.050 g in sufficient water to produce 100 ml. To 10 ml add 5 ml of ammonium molybdate/vanadate TS, mix and allow to stand for 5 minutes; any yellow colour produced is not more intense than that of a reference solution prepared similarly using 10 ml of phosphate standard (5 µg/ml) TS.

Free prednisolone and other related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (15 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture prepared as follows: weigh 1.36 g of potassium dihydrogen phosphate R and 0.60 g of hexylamine R, transfer to a 250-ml conical flask, mix, and allow to stand for 10 minutes, and then dissolve in 185 ml of water. Add 65 ml of acetonitrile R, mix, and filter.

   Prepare the following solutions in the mobile phase: solution (A) 2.5 mg of Prednisolone sodium phosphate per ml; solution (B) 2.5 mg of prednisolone sodium phosphate RS and 2.5 mg of prednisolone RS per ml, dilute 1.0 ml of this solution to 25 ml with the mobile phase; and for solution (C) dilute 1.0 ml of solution A to 50 ml with the mobile phase.

   Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

   Inject 20 µl of solution B. Adjust the sensitivity of the system so that the heights of the principal peaks in the chromatogram obtained with solution B are 70–90% of the full scale of the recorder. The retention times for prednisolone sodium phosphate are about 6.5 minutes, and for prednisolone about 8.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone sodium phosphate and prednisolone is not less than 4.5.

   Inject alternately 20 µl each of solutions A and C. Continue the chromatography for 3 times the retention time of the principal peak.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the contents of free prednisolone and other related substances as a percentage. In the chromatogram obtained from solution A, the area of any peak, other than the principal peak, is not greater than that of the principal peak obtained with solution C (2.0%), and not more than one such peak has an area greater than half the area of the principal peak obtained with solution C (1.0%). The sum of the areas of all the peaks, other than the principal peak, is not greater than 1.5 times the area of the principal peak obtained with solution C (3.0%). Disregard any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and methanol R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 10 mg of Prednisolone sodium phosphate per ml, and (B) 0.20 mg of prednisolone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 5 minutes, spray with a solution of 3 g of zinc chloride R in 10 ml of methanol R, heat at about 125 °C for 1 hour, and examine the chromatogram in ultraviolet light (365 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (2.0%).

Assay. Dissolve about 0.1 g, accurately weighed, in sufficient water to produce 100 ml. Dilute 5 ml to 250 ml with water and measure the absorbance of this solution in a 1-cm layer at the maximum at about 247 nm.
Calculate the percentage content of $C_{21}H_{27}Na_2O_8P$ using the absorptivity value of 31.2 ($A^{1\%}_{1\,cm} = 312$).

Additional requirement for sterile non-injectable Prednisolone sodium phosphate

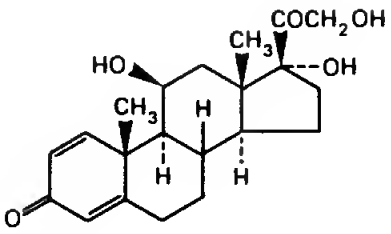
Complies with 3.2.1 Test for sterility of non-injectable preparations.

PREDNISOLONUM
PREDNISOLONE

Molecular formula. $C_{21}H_{28}O_5$

Relative molecular mass. 360.5

Graphic formula.



Chemical name. 11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione; CAS Reg. No. 50-24-8.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 1300 parts of water and in 30 parts of dehydrated ethanol R; soluble in methanol R and dioxan R.

Category. Adrenocortical steroid.

Storage. Prednisolone should be kept in a tightly closed container, protected from light.

Additional information. Prednisolone is hygroscopic; it has a melting temperature of about 230 °C with decomposition.

Requirements

Definition. Prednisolone contains not less than 97.0% and not more than 102.0% of $C_{21}H_{28}O_5$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone RS or with the *reference spectrum* of prednisolone.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of formamide R and 90 volumes of acetone R to impregnate the plate, dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the

solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml and (B) 2.5 mg of prednisolone RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with sulfuric acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +96$ to + 103 °.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the test substance per ml and (B) 0.30 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated and heat at 105 °C for 10 minutes; cool, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

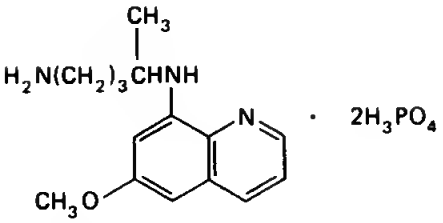
Assay. Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 242 nm. Calculate the amount of $C_{21}H_{28}O_5$ in the substance being tested by comparison with prednisolone RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.44 ± 0.02 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

PRIMAQUINI DIPHOSPHAS
PRIMAQUINE DIPHOSPHATE

Molecular formula. $C_{15}H_{21}N_3O,2H_3PO_4$.

Relative molecular mass. 455.3

Graphic formula.



Chemical name. 8-[(4-Amino-1-methylbutyl)amino]-6-methoxyquinoline phosphate (1:2); N^4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine phosphate (1:2); CAS Reg. No. 63-45-6.

Description. An orange-red, crystalline powder; odourless or almost odourless.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS, and ether R.

Category. Antimalarial.

Storage. Primaquine diphosphate should be kept in a well-closed container, protected from light.

Requirements

Definition. Primaquine diphosphate contains not less than 98.0% and not more than 102.0% of $C_{15}H_{21}N_3O,2H_3PO_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from primaquine diphosphate RS or with the *reference spectrum* of primaquine diphospate.

B. Dissolve 10 mg in 5 ml of water and add 1 ml of ceric ammonium sulfate/nitric acid TS; a deep violet colour is immediately produced (distinction from chloroquine).

C. To 1 ml of a 20 mg/ml solution add 3 ml of nitric acid (~130 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

D. Melting temperature, about 202 °C.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution, 2.5–3.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 3 volumes of dimethylamine/ethanol TS, 4 volumes of acetone R and 5 volumes of chloroform R as the mobile phase. To 5 ml of a solution containing 20 mg of the test substance per ml add 5 ml of chloroform R and 0.5 ml of ammonia (~35 g/l) TS and shake. Separate the chloroform layer, filter and apply 5 µl of this solution to the plate. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm). Only a single fluorescent spot is obtained.

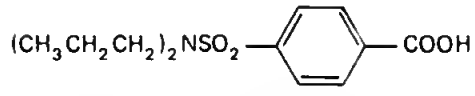
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.9 g, accurately weighed, and 50 ml of hydrochloric acid (~70 g/l) TS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 45.53 mg of $C_{15}H_{21}N_3O,2H_3PO_4$.

PROBENECIDUM
PROBENECID

Molecular formula. $C_{13}H_{19}NO_4S$

Relative molecular mass. 285.4

Graphic formula.



Chemical name. *p*-(Dipropylsulfamoyl)benzoic acid; 4-[(dipropylamino)sulfonyl]benzoic acid; CAS Reg. No. 57-66-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 25 parts of ethanol (~750 g/l) TS and in 12 parts of acetone R; soluble in dilute solutions of alkali hydroxides.

Category. Antigout drug.

Storage. Probenecid should be kept in a well-closed container.

Requirements

Definition. Probenecid contains not less than 98.0% and not more than 101.0% of $C_{13}H_{19}NO_4S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from probenecid RS or with the *reference spectrum* of probenecid.

B. See the test described below under "Related substances". The principal spot obtained with solution A (first application) corresponds in position, appearance and intensity with that obtained with solution B.

C. Melting temperature, about 199 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Heat 2.0 g with 100 ml of carbon-dioxide-free water R on a water-bath for 30 minutes, cool and filter. Add 0.15 ml of phenolphthalein/ethanol TS to the filtrate and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS; not more than 0.5 ml of the titrant is required to obtain the midpoint of the indicator.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture

of 15 volumes of 1-propanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Prepare the 3 following solutions in a mixture of 1 volume of ammonia (~17 g/l) TS and 9 volumes of ethanol (~750 g/l) TS containing (A) 10 mg of the test substance per ml, (B) 10 mg of probenecid RS per ml, and (C) 0.050 mg of the test substance per ml. Apply to the plate 1 µl of each of solutions A and B. Apply separately 20 µl of each of solutions A and C. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A (second application), other than the principal spot, is not more intense than that obtained with solution C.

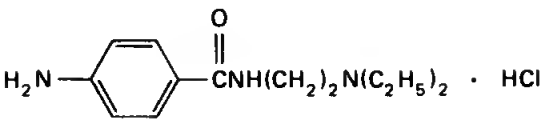
Assay. Dissolve about 1.0 g, accurately weighed, in 50 ml of neutralized ethanol TS and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using bromothymol blue/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 28.54 mg of $C_{13}H_{19}NO_4S$.

PROCAINAMIDI HYDROCHLORIDUM
PROCAINAMIDE HYDROCHLORIDE

Molecular formula. $C_{13}H_{21}N_3O,HCl$

Relative molecular mass. 271.8

Graphic formula.



Chemical name. *p*-Amino-*N*-[2-(diethylamino)ethyl]benzamide monohydrochloride; 4-amino-*N*-[2-(diethylamino)ethyl]benzamide monohydrochloride; CAS Reg. No. 614-39-1.

Description. A white to yellowish white, crystalline powder; odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Antiarrhythmic.

Storage. Procainamide hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Procainamide hydrochloride is hygroscopic. Even in the absence of light, Procainamide hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Procainamide hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{13}H_{21}N_3O$,HCl, calculated with reference to the dried substance.

Identity tests

A. Dissolve 1 g in 10 ml of water, add 10 ml of sodium hydroxide (~200 g/l) TS, and extract with 10 ml of chloroform R. To the extract add 10 ml of toluene R, dry over anhydrous sodium sulfate R, and filter. Mix the filtrate with 5 ml of anhydrous pyridine R, add 1 ml of benzoyl chloride R drop by drop, heat on a water-bath for 30 minutes, and pour into a mixture of 50 ml of water and 50 ml of sodium hydroxide (~200 g/l) TS. Extract with 10 ml of ether R, wash the extract with 20 ml of water, dilute with 30 ml of ether R, and allow to crystallize. Recrystallize from ethanol (~375 g/l) TS; melting temperature, about 185 °C (benzoyl procainamide).

B. Dissolve 0.1 g in 2 ml of water and add 2 ml of potassium ferrocyanide (45 g/l) TS. Add a few drops of hydrochloric acid (~70 g/l) TS to acidify slightly and heat; a light green precipitate is produced.

C. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 165–169 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C: it loses not more than 3.0 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 5.0–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 4 volumes of 1-butanol R, 1 volume of glacial acetic acid R, and 2 volumes of water as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.25 g, accurately weighed, in 5 ml of acetic anhydride R and 15 ml of glacial acetic acid R1. Heat the solution until boiling. Add 20 ml of dioxan R and 20 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.18 mg of $C_{13}H_{21}N_3O,HCl$.

Additional requirements for Procainamide hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.35 IU of endotoxin RS per mg.

PROCAINI BENZYLPENICILLINUM

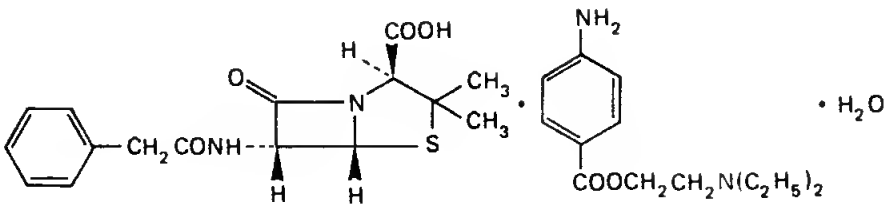
PROCAINE BENZYLPENICILLIN

Procaine benzylpenicillin (non-injectable)
Procaine benzylpenicillin, sterile

Molecular formula. $C_{16}H_{18}N_2O_4S,C_{13}H_{20}N_2O_2,H_2O$

Relative molecular mass. 588.7

Graphic formula.



Chemical name. 2-(Diethylamino)ethyl *p*-aminobenzoate compound with (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid (1:1) monohydrate; 2-(diethylamino)ethyl 4-aminobenzoate compound with [2*S*-(2α,5α,6β)]-3,3-dimethyl-7-oxo-6-[(phenyl-acetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1:1) monohydrate; CAS Reg. No. 6130-64-9 (monohydrate).

Other names. Penicillin G procaine, Procaine penicillin.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Slightly soluble in water; soluble in ethanol (~750 g/l) TS.

Category. Antibacterial drug.

Storage. Procaine benzylpenicillin should be kept in a tightly closed container and protected from light.

Labelling. The designation sterile Procaine benzylpenicillin indicates that the substance complies with the additional requirements for sterile Procaine benzylpenicillin and may be used for parenteral administration or for other sterile applications.

Additional information. Solutions of Procaine benzylpenicillin are dextrorotatory. It is readily decomposed by acids, alkalis, and oxidizing agents. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Procaine benzylpenicillin contains not less than 96.0% and not more than 100.5% of total penicillins calculated as $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$ and not less than 38.5% and not more than 41.5% of $C_{13}H_{20}N_2O_2$, both calculated with reference to the anhydrous substance.

Identity tests

A. To 2 mg in a test-tube add 0.05 ml of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is almost colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains almost colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS and mix; the solution is almost colourless, but after a few minutes the colour changes to yellow-brown. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

B. Dissolve 10 mg in 10 ml of water and add 0.5 ml of neutral red/ethanol TS. Add sufficient sodium hydroxide (0.01 mol/l) VS to give a permanent orange colour and then add 1.0 ml of penicillinase TS; the colour changes rapidly to red.

C. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a bright, orange-red precipitate.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; the water content is not less than 28 mg/g and not more than 42 mg/g.

pH value. pH of a saturated solution containing about 3.0 g in 10 ml of carbon-dioxide-free water R, 5.0–7.5.

Assay

For total penicillins. Dissolve about 0.045 g, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10.0 ml of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (solution A). To the second tube add 10.0 ml of water and mix (solution B).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 314 nm against a solvent cell containing a mixture of 2.0 ml of water and 10.0 ml of imidazole/mercuric chloride TS for solution A and water for solution B.

From the difference between the absorbance of solution A and that of solution B, calculate the amount of $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$ in the substance being tested by comparison with 0.050 g of benzylpenicillin sodium RS similarly and concurrently examined, taking into account that each mg of benzylpenicillin sodium RS ($C_{16}H_{17}N_2NaO_4S$) is equivalent to 1.601 mg of $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.62 ± 0.03.

For procaine. Dissolve about 0.5 g, accurately weighed, in 10 ml of water, add 5 ml of sodium carbonate (75 g/l) TS and extract with four successive quantities, each of 25 ml of chloroform R, filter the chloroform extracts, and evaporate to a small volume on a water-bath. Add 20.0 ml of hydrochloric acid (0.1 mol/l) VS and distil off the remaining chloroform. Cool, add 0.25 ml of methyl red/ethanol TS and titrate with sodium hydroxide (0.1 mol/l) VS. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 23.63 mg of $C_{13}H_{20}N_2O_2$.

Additional requirements for Procaine Benzylpenicillin for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

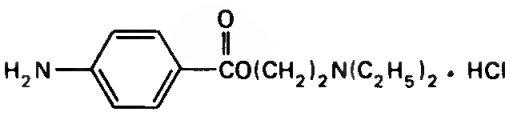
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

PROCAINI HYDROCHLORIDUM
PROCAINE HYDROCHLORIDE

Molecular formula. $C_{13}H_{20}N_2O_2,HCl$

Relative molecular mass. 272.8

Graphic formula.



Chemical name. 2-(Diethylamino)ethyl *p*-aminobenzoate monohydrochloride; 2-(diethylamino)ethyl 4-aminobenzoate monohydrochloride; CAS Reg. No. 51-05-8.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Soluble in 1 part of water and in 25 parts of ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Local anaesthetic.

Storage. Procaine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Procaine hydrochloride causes local numbness after being placed on the tongue. Even in the absence of light, Procaine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Procaine hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{13}H_{20}N_2O_2$,HCl, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from procaine hydrochloride RS or with the *reference spectrum* of procaine hydrochloride.

B. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a vivid red precipitate.

C. Dissolve 0.05 g in 5 ml of water, add 5 drops of sulfuric acid (~100 g/l) TS and 2 drops of potassium permanganate (0.02 mol/l) VS; the violet colour produced disappears quickly.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 154–158 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Sulfated ash. Not more than 1.5 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 5.0–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 80 volumes of dibutyl ether R, 16 volumes of hexane R, and 4 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 0.10 g of the test substance per ml and (B) 0.050 mg

of 4-aminobenzoic acid R per ml. After removing the plate from the chromatographic chamber, allow it to dry at 105 °C for 10 minutes, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. The principal spot remains at the point of application.

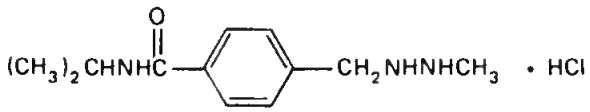
Assay. Carry out the assay as described under 2.7 Nitrite titration; dissolve about 0.5 g, accurately weighed, in 50 ml of hydrochloric acid (~70 g/l) TS, add 0.1 g of potassium bromide R, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 27.28 mg of $C_{13}H_{20}N_2O_2$,HCl.

PROCARBAZINI HYDROCHLORIDUM
PROCARBAZINE HYDROCHLORIDE

Molecular formula. $C_{12}H_{19}N_3O$,HCl

Relative molecular mass. 257.8

Graphic formula.



Chemical name. *N*-Isopropyl-α-(2-methylhydrazino)-*p*-toluamide monohydrochloride; *N*-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide monohydrochloride; CAS Reg. No. 366-70-1.

Description. A white to yellowish, crystalline powder.

Solubility. Soluble in water and methanol R; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Procarbazine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Procarbazine hydrochloride melts at about 223 °C with decomposition. Even in the absence of light, Procarbazine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. CAUTION: Procarbazine hydrochloride

must be handled with care, avoiding contact with the skin and inhalation of airborne particles. Wear rubber gloves while handling this substance.

Requirements

Definition. Procarbazine hydrochloride contains not less than 98.5% and not more than 100.5% of $C_{12}H_{19}N_3O$,HCl, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from procarbazine hydrochloride RS or with the *reference spectrum* of procarbazine hydrochloride.

B. A 0.10 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.05 g/ml solution, 3.0–4.5.

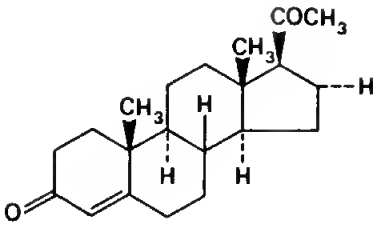
Assay. Dissolve about 0.125 g, accurately weighed, in a mixture of 5 ml of formic acid (~1080 g/l) TS and 20 ml of glacial acetic acid R1, add 5 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.78 mg of $C_{12}H_{19}N_3O$,HCl.

PROGESTERONUM
PROGESTERONE

Molecular formula. $C_{21}H_{30}O_2$

Relative molecular mass. 314.5

Graphic formula.



Chemical name. Pregn-4-ene-3,20-dione; CAS Reg. No. 57-83-0.

Description. Colourless crystals or a white to slightly yellowish white, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 8 parts of ethanol (~750 g/l) TS.

Category. Progestational steroid.

Storage. Progesterone should be kept in a well-closed container, protected from light.

Additional information. Progesterone may exist in 2 polymorphic forms, one of which melts at about 130 °C, the other at about 121 °C.

Requirements

Definition. Progesterone contains not less than 97.0% and not more than 102.0% of $C_{21}H_{30}O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A or test B may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from progesterone RS or with the *reference spectrum* of progesterone. If the spectrum obtained from the solid state of the test substance is not concordant with the spectrum obtained from the reference substance, compare the spectra using solutions in chloroform R containing 30 mg/ml and a path length of 0.2 mm.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 10 volumes of propylene glycol R and 90 volumes of acetone R to impregnate the plate,

dipping it about 5 mm beneath the surface of the liquid. After the solvent has reached the height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. As the mobile phase, use a mixture of 50 volumes of cyclohexane R and 50 volumes of light petroleum R. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 1.0 mg of the test substance per ml and (B) 1.0 mg of progesterone RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, spray with 4-toluenesulfonic acid/ethanol TS, and then heat at 120 °C for 10 minutes. Allow to cool and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R; $[\alpha]_D^{20\,°C} = +186$ to $+196$ °.

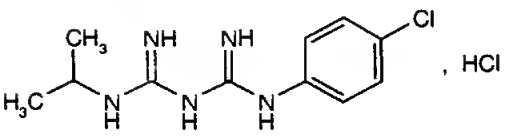
Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 2 parts of chloroform R and 1 part of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 1 volume of ethanol (~750 g/l) TS and 1 volume of chloroform R containing (A) 10 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 240 nm. Calculate the amount of $C_{21}H_{30}O_2$ in the substance being tested by comparison with progesterone RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.54 ± 0.03.

PROGUANILI HYDROCHLORIDUM
PROGUANIL HYDROCHLORIDE

$C_{11}H_{16}ClN_5,HCl$



Relative molecular mass. 290.2.

Chemical name. 1-(*p*-Chlorophenyl)-5-isopropylbiguanide hydrochloride; CAS Reg. No. 637-32-1.

Description. A white, crystalline powder.

Solubility. Slightly soluble in water, more soluble in hot water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antimalarial drug.

Storage. Proguanil hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Proguanil hydrochloride contains not less than **99.0%** and not more than **101.0%** of $C_{11}H_{16}ClN_5,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from proguanil hydrochloride RS or with the *reference spectrum* of proguanil hydrochloride.

B. Dissolve about 0.1 g in 10 ml of water, add 5 drops of potassium ferrocyanide (45 g/l) TS; a white precipitate is produced. Add 10–15 drops of nitric acid (~130 g/l) TS; the precipitate dissolves.

C. Dissolve about 0.1 g in 10 ml of water, add 3 drops of copper(II) sulfate (160 g/l) TS and 1.0 ml of ammonia (~100 g/l) TS, shake, add 5 ml of toluene R, and shake again; a violet colour is produced in the toluene layer.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

Acidity or alkalinity. To 35 ml of water maintained at a temperature of about 65 °C add 0.20 ml of methyl red/methylthioninium chloride TS, neutralize with sodium hydroxide (0.01 mol/l) VS or hydrochloric acid (0.01 mol/l) VS, add 0.4 g of Proguanil hydrochloride, and stir until dissolved; the resulting solution is not acidic and requires for neutralization not more than 0.2 ml of hydrochloric acid (0.01 mol/l) VS.

Chloraniline. For solution A, dissolve 0.10 g in 1 ml of hydrochloric acid (~70 g/l) TS, and add sufficient water to produce 20 ml. Cool to 5 °C, add 1 ml of sodium nitrite (35 g/l) TS, allow to stand at 5 °C for 5 minutes, add 2 ml of ammonium sulfamate (50 g/l) TS, shake, and allow to stand for 10 minutes. Add 2 ml of freshly prepared *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS, dilute to 50 ml with water, and allow to stand for 30 minutes. For solution B, treat similarly 20 ml of a solution containing 1.25 µg of chloraniline R per ml. Any magenta colour produced in solution A is not more intense than that produced in solution B (250 µg/g).

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 5.0 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a solution prepared as follows: dissolve 1.88 g of sodium hexanesulfonate R in 1000 ml of a mixture of 12 volumes of methanol R, 8 volumes of water, and 0.1 volume of glacial acetic acid R.

Prepare the following solutions in the mobile phase: solution (A) 1.0 µg of Proguanil hydrochloride per ml; and solution (B) 0.10 mg of Proguanil hydrochloride per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Inject alternately 10 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. The sum of the areas of any peaks, other than the principal peak, in the chromatogram obtained from solution B is not greater than that of the principal peak obtained with solution A (1%).

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically.

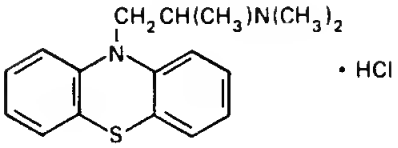
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 14.51 mg of $C_{11}H_{16}ClN_5$,HCl.

PROMETHAZINI HYDROCHLORIDUM
PROMETHAZINE HYDROCHLORIDE

Molecular formula. $C_{17}H_{20}N_2S$,HCl

Relative molecular mass. 320.9

Graphic formula.



Chemical name. 10-[2-(Dimethylamino)propyl]phenothiazine monohydrochloride; *N,N,*α-trimethyl-10*H*-phenothiazine-10-ethanamine monohydrochloride; CAS Reg. No. 58-33-3.

Other name. Diprazinum.

Description. A white or faintly yellowish, crystalline powder; odourless or almost odourless.

Solubility. Very soluble in water; freely soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiemetic drug.

Storage. Promethazine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. On prolonged exposure to air, Promethazine hydrochloride slowly oxidizes and acquires a blue colour. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Promethazine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{17}H_{20}N_2S,HCl$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 20 mg in 5 ml of water and add 0.05 g of lead(IV) oxide R; no red coloration is observed in the supernatant liquid but it slowly turns bluish.

B. Dissolve 0.25 g in 25 ml of water and add slowly, with stirring, 25 ml of trinitrophenol (7 g/l) TS. Allow the mixture to stand for 10 minutes, collect the precipitate and wash with a small quantity of water; melting temperature after recrystallization from ethanol (~750 g/l) TS and drying, about 160 °C with decomposition (picrate).

C. Dissolve 0.05 g in 5 ml of water and add 2 ml of nitric acid (~1000 g/l) TS; a dark red colour is produced, which turns yellowish on standing. The solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Solution in chloroform. A solution of 1.0 g in 10 ml of chloroform R is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water, 3.5–5.0.

Related impurities. Carry out, in subdued light, the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 85 volumes of hexane R, 10 volumes of acetone R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 freshly prepared solutions in a mixture of 95 volumes of methanol R and 5 volumes of diethylamine R containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 200 ml of acetone R, add 10 ml of mercuric acetate/acetic acid TS and 3 ml of methyl orange/acetone TS,

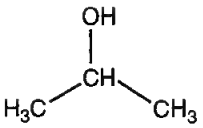
and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 32.09 mg of $C_{17}H_{20}N_2S,HCl$.

Additional requirements for Promethazine hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 5.0 IU of endotoxin RS per mg.

2-PROPANOLUM
2-PROPANOL



C_3H_8O

Relative molecular mass. 60.10

Chemical name. Isopropyl alcohol; 2-propanol; CAS Reg. No. 67-63-0.

Description. A colourless, clear, and mobile liquid; odour, characteristic.

Miscibility. Miscible with water, ethanol (~750 g/l) TS, and ether R.

Category. Solvent; antiseptic.

Storage. 2-Propanol should be kept in a tightly closed container, in a cool place.

Additional information. 2-Propanol is volatile and flammable. Boiling range, 81–83 °C.

Requirements

Identity tests

A. Mix 1 ml with 9 ml of water. To 1 ml of this solution add 2 ml of mercuric sulfate TS and heat to boiling; a white to yellowish white precipitate is produced.

B. Heat gently 1 ml with 3 ml of potassium dichromate (100 g/l) TS and 1 ml of sulfuric acid (~1760 g/l) TS; acetone, perceptible by its odour, is evolved.

Refractive index. $n_D^{20} = 1.376 - 1.378$.

Relative density. $d_{20}^{20} = 0.783 - 0.787$.

Nonvolatile residue. Evaporate 50 ml to dryness on a water-bath, heat at 105 °C for 1 hour, and weigh; not more than 2.5 mg (0.005%).

Acidity. To 50 ml add 100 ml of carbon-dioxide-free water R and 2 drops of phenolphthalein/ethanol TS, and titrate with carbonate-free sodium hydroxide (0.02 mol/l) VS to a pink colour that persists for 30 seconds; not more than 0.7 ml of carbonate-free sodium hydroxide (0.02 mol/l) VS is required.

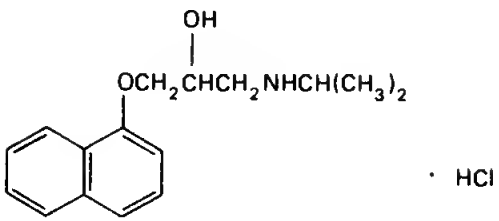
Aldehydes and ketones. Transfer 25 ml to a comparison tube, add 25 ml of water and 50 ml of hydroxylamine hydrochloride TS, mix, and allow to stand for 5 minutes. Titrate with sodium hydroxide (0.1 mol/l) VS until the colour matches that of 50 ml of hydroxylamine hydrochloride TS placed in a similar tube and viewed down the vertical axis; not more than 2.0 ml of sodium hydroxide (0.1 mol/l) VS is required.

PROPRANOLOLI HYDROCHLORIDUM
PROPRANOLOL HYDROCHLORIDE

Molecular formula. $C_{16}H_{21}NO_2,HCl$

Relative molecular mass. 295.8

Graphic formula.



Chemical name. (±)-1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride; (±)-1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol hydrochloride; CAS Reg. No. 318-98-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in water and in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiadrenergic.

Storage. Propranolol hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Propranolol hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{16}H_{21}NO_2$,HCl, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from propranolol hydrochloride RS or with the *reference spectrum* of propranolol hydrochloride.

B. The absorption spectrum of a 20 µg/ml solution in methanol R, when observed between 230 nm and 350 nm, is qualitatively similar to that of a 20 µg/ml solution in methanol R of propranolol hydrochloride RS (maxima occur at about 290 nm, 306 nm, and 319 nm). The absorbances of the solutions at their respective maxima do not differ from each other by more than 3%. The absorbances of a 1-cm layer at those wavelengths are about 0.42, 0.25 and 0.15 (preferably use 2-cm cells for the measurement and calculate the absorbances of 1-cm layers).

C. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 161–165 °C.

Specific optical rotation. Use a 0.10 g/ml solution; the substance is optically inactive.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

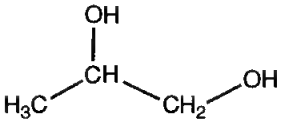
Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 10 mg/ml solution, 5.0–6.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 140 volumes of dichloroethane R, 60 volumes of methanol R, 2.5 volumes of water, and 2.5 volumes of anhydrous formic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in chloroform R containing (A) 10 mg of the test substance per ml and (B) 0.050 mg of the test substance per ml. Develop the plate for a distance of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.6 g, accurately weighed, in 50 ml of glacial acetic acid R1, and add 10 ml of mercuric acetate/acetic acid TS, warming slightly if necessary to effect solution. Cool and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 29.58 mg of $C_{16}H_{21}NO_2,HCl$.

PROPYLENEGLYCOLUM
PROPYLENE GLYCOL



$C_3H_8O_2$

Relative molecular mass. 76.09

Chemical name. 1,2-Propanediol; CAS Reg. No. 57-55-6.

Description. A colourless, clear, and viscous liquid; odourless.

Miscibility. Miscible with water, ethanol (~750 g/l) TS, and chloroform R.

Category. Solvent; humectant.

Storage. Propylene glycol should be kept in a tightly closed container.

Additional information. Propylene glycol is hygroscopic. Boiling range, 185–189 °C.

Requirements

Identity test

Dissolve 0.1 ml in sufficient water to produce 100 ml, dilute 1 ml to 10 ml, and place 0.5 ml of this solution in a test-tube. Cool in ice, add 5 ml of a cooled mixture of 10 ml of water and 90 ml of sulfuric acid (~1760 g/l) TS, heat on a water-bath at 70 °C for 10 minutes, and cool again. Add 0.2 ml of triketohydrindene/sodium metabisulfite TS; a violet colour slowly appears.

Refractive index. $n_D^{20} = 1.431 - 1.433$.

Relative density. $d_{20}^{20} = 1.035 - 1.040$.

Heavy metals. Use 4 ml for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 5 µg/g.

Clarity and colour of solution. Propylene glycol should be clear and colourless.

Sulfated ash. Use 50 g; the residue weighs not more than 5 mg (0.1 mg/g).

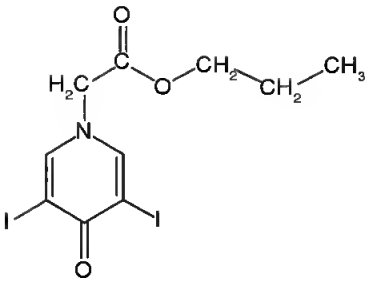
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 5 g; the water content is not more than 2.0 mg/g.

Acidity. To 10 ml add 40 ml of water and 0.1 ml of bromothymol blue/ethanol TS; the solution is greenish yellow. Titrate with sodium hydroxide (0.1 mol/l) VS; not more than 0.05 ml is required to obtain the midpoint of the indicator (blue).

Oxidizing substances. To 10 ml add 5 ml of water, 2 ml of potassium iodide (80 g/l) TS, and 2 ml of sulfuric acid (~100 g/l) TS, and allow to stand in a stoppered flask protected from light for 15 minutes. Titrate with sodium thiosulfate (0.05 mol/l) VS, using starch TS as indicator; not more than 0.2 ml is required.

Reducing substances. To 1 ml add 1 ml of ammonia (~100 g/l) TS and heat in a water-bath at 60 °C for 5 minutes; the solution is yellow. Without delay add 0.15 ml of silver nitrate (0.1 mol/l) VS and allow to stand for 5 minutes; the colour and aspect of the solution remain unchanged.

PROPYLIODONUM
PROPYLIODONE



$C_{10}H_{11}I_2NO_3$

Relative molecular mass. 447.0

Chemical name. Propyl 3,5-diiodo-4-oxo-1(4*H*)-pyridineacetate; CAS Reg. No. 587-61-1.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Radiocontrast medium.

Storage. Propyliodone should be kept in a tightly closed container, protected from light.

Requirements

Propyliodone contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{10}H_{11}I_2NO_3$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 20 µg/ml solution in dehydrated ethanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 239 nm and 281 nm; the absorbances of a 1-cm layer at those wavelengths are about 0.64 and 0.52, respectively.

B. Heat 0.1 g with a few drops of sulfuric acid (~1760 g/l) TS in a suitable crucible; violet vapours are evolved.

Melting range. 187–190 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Halides. Shake 2.4 g with 30 ml of water for 15 minutes and filter. To 10 ml of the filtrate add 1 ml of nitric acid (~130 g/l) TS, 2 ml of sodium nitrite (1 g/l) TS, and 2 ml of chloroform R, shake well, and centrifuge. To serve as a reference solution, treat similarly 2 ml of iodide standard (20 µg I/ml) TS with 8 ml of water. The content of halides, expressed as iodides, does not produce a solution with any red-violet colour more intense than that of the reference solution.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Dissolve 1 g in 40 ml of hot 1-propanol R, previously neutralized using phenolphthalein/ethanol TS, cool, and allow to stand for 15 minutes with frequent shaking. Filter, wash the residue with neutralized 1-propanol R, filter again, and combine the filtrate and the wash liquids. Titrate with sodium hydroxide (0.05 mol/l) VS, using phenolphthalein/ethanol TS as indicator, until a pink colour persists for 15 seconds; not more than 0.15 ml of sodium hydroxide (0.05 mol/l) VS is required.

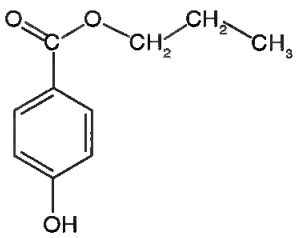
Assay. Carry out the combustion as described under 2.4 Oxygen flask method for iodine, using about 15 mg, accurately weighed. Titrate the liberated iodine with sodium thiosulfate (0.02 mol/l) VS.

Each ml of sodium thiosulfate (0.02 mol/l) VS is equivalent to 0.7450 mg of $C_{10}H_{11}I_2NO_3$.

Additional requirement for Propyliodone for parenteral use

Complies with the monograph for "Parenteral preparations".

PROPYLIS HYDROXYBENZOAS
PROPYL HYDROXYBENZOATE



$C_{10}H_{12}O_3$

Relative molecular mass. 180.2

Chemical name. Propyl *p*-hydroxybenzoate; propyl 4-hydroxybenzoate; CAS Reg. No. 94-13-3.

Other name. Propylparaben.

Description. Colourless crystals or a white, crystalline powder; odourless or with a faintly aromatic odour.

Solubility. Very slightly soluble in water; slightly soluble in boiling water; freely soluble in ethanol (~750 g/l) TS and ether R.

Category. Antimicrobial preservative.

Storage. Propyl hydroxybenzoate should be kept in a well-closed container.

Additional information. Propyl hydroxybenzoate is normally used in combination with other hydroxybenzoates.

Requirements

Propyl hydroxybenzoate contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{10}H_{12}O_3$, calculated with reference to the dried substance.

Identity tests

A. Complies with the test under "Melting range".

B. To 0.5 g add 5 ml of sodium hydroxide (~80 g/l) TS and heat in a water-bath for 5 minutes. After cooling, add 6 ml of sulfuric acid (~190 g/l) TS, collect

the precipitate on a filter, wash thoroughly with a small amount of water, and dry over silica gel, desiccant, R. Melting temperature, about 214 °C.

Melting range. 96–99 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 2 hours; it loses not more than 5.0 mg/g.

Acidity. Dissolve 0.2 g in 5 ml of ethanol (~750 g/l) TS, add 5 ml of carbon-dioxide-free water R, and titrate with sodium hydroxide (0.1 mol/l) VS, using 0.1 ml of bromocresol green/ethanol TS as indicator; not more than 0.1 ml is required to obtain the midpoint of the indicator (green).

Assay. Place about 80 mg, accurately weighed, in a ground-glass-stoppered flask, add 25 ml of sodium hydroxide (~80 g/l) TS, and boil gently under a reflux condenser for 30 minutes. Allow to cool, add 25 ml of potassium bromate (0.0333 mol/l) VS, 5 ml of potassium bromide (125 g/l) TS, and 40 ml of glacial acetic acid R. Cool in ice-water and add 10 ml of hydrochloric acid (~420 g/l) TS. Stopper the flask immediately and allow to stand for 15 minutes. Add 30 ml of potassium iodide (80 g/l) TS, close the flask and mix. Titrate with sodium thiosulfate (0.1 mol/l) VS, using 2 ml of starch TS as indicator, added towards the end of the titration. Repeat the procedure without the Propyl hydroxybenzoate being examined and make any necessary corrections.

One volume of sodium thiosulfate (0.1 mol/l) VS corresponds to two volumes of potassium bromate (0.0333 mol/l) VS. Each ml of potassium bromate (0.0333 mol/l) VS is equivalent to 6.007 mg of $C_{10}H_{12}O_3$.

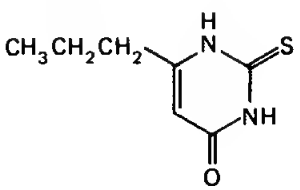
PROPYLTHIOURACILUM

PROPYLTHIOURACIL

Molecular formula. $C_7H_{10}N_2OS$

Relative molecular mass. 170.2

Graphic formula.



Chemical name. 6-Propyl-2-thiouracil; 2,3-dihydro-6-propyl-2-thioxo-4(1*H*)-pyrimidinone; 6-propyl-2-thio-2,4(1*H*,3*H*)-pyrimidinedione; CAS Reg. No. 51-52-5.

Description. Colourless or pale cream-coloured crystals or a white or cream-coloured, crystalline powder; odourless.

Solubility. Very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Antithyroid substance.

Storage. Propylthiouracil should be kept in a well-closed container, protected from light.

Additional information. Propylthiouracil has a bitter taste.

Requirements

Definition. Propylthiouracil contains not less than 98.0% and not more than 100.5% of $C_7H_{10}N_2OS$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of propylthiouracil.

B. Dissolve 0.05 g in 5 ml of boiling water and to the hot solution add a freshly prepared solution of 20 mg of hydroxylamine hydrochloride R and 0.04 g of anhydrous sodium carbonate R in 5 ml of water, to which 0.4 ml of sodium nitroprusside (45 g/l) TS has been added; a greenish blue colour is produced.

C. To 25 mg add bromine TS1, drop by drop, until the substance is completely dissolved. Warm until the colour is discharged, cool, and add 10 ml of barium hydroxide (15 g/l) TS; a white precipitate is produced (distinction from thiouracil, which yields a white precipitate that turns purple within 1 minute).

D. Melting temperature, about 220 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Thiourea. Boil 0.50 g with 50 ml of water under a reflux condenser until dissolved and dilute 5 ml of the hot solution to 50 ml with water. Place 10 ml of this solution in a test-tube and add to it 1 ml of thiourea (0.1 g/l) TS. Cool the remainder of the hot solution, filter, and place 10 ml of the filtrate in a second test-tube, to serve as a reference. To each tube add 0.5 g of sodium acetate R and 5 ml of silver nitrate (0.1 mol/l) VS and heat in a water-bath for 5 minutes. The colour produced in the test solution, when viewed transversely against a white background, is not more intense than that of the reference solution when compared as described under 1.11 Colour of liquids.

Assay. Transfer about 0.3 g, accurately weighed, to a 500-ml flask and add 30 ml of water. Add from a burette about 30 ml of sodium hydroxide (0.1 mol/l) VS, heat to boiling, and shake the flask until solution is complete. Wash down any particles on the wall of the flask with a small volume of water, then add about 50 ml of silver nitrate (0.1 mol/l) VS while mixing, and boil gently for 5 minutes. Add 1–2 ml of bromothymol blue/ethanol TS, and continue to titrate with sodium hydroxide (0.1 mol/l) VS until a permanent blue-green colour is produced. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 8.51 mg of $C_7H_{10}N_2OS$.

PROTAMINI SULFAS
PROTAMINE SULFATE

Chemical name. Protamine sulfate; CAS Reg. No. 9009-65-8.

Description. A white or almost white powder; hygroscopic.

Solubility. Soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

Category. Drug affecting blood coagulation.

Storage. Protamine sulfate should be kept in a tightly closed and tamper-evident container.

Labelling. The designation Protamine sulfate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

Additional information. Protamine sulfate binds with heparin in solution, inhibiting its anticoagulant activity. It is prepared in conditions designed to minimize the risk of microbial contamination.

Requirements

Definition. Protamine sulfate is a mixture of sulfates of purified proteins extracted from the sperm or roe of fish usually belonging to the family *Clupidae* and *Salmonidae*.

The quantity of **1 mg** of Protamine sulfate precipitates not less than **100 IU** of heparin sodium activity, calculated with reference to the dried substance.

Identity tests

A. Use a 10 mg/ml solution in hydrochloric acid (0.1 mol/l) VS. Measure the optical rotation and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = 65°$ to $-85°$.

B. Dissolve 0.1 g in 5 ml of water, add 4.5 ml of water, 1.0 ml of sodium hydroxide (~80 g/l) TS, and 2.0 ml of 1-naphthol TS1. Cool the mixture to 5 °C and add 0.5 ml of sodium hypobromite TS; an intense red colour is produced.

C. Dissolve 0.04 g in 2 ml of water and heat in a water-bath at 60 °C. Add 0.1 ml of mercuric sulfate TS and mix; no precipitate is formed. Cool the mixture in an ice-bath; a precipitate is formed.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfates. Transfer 0.15 g to a beaker and dissolve in 15 ml of water. Add 5 ml of hydrochloric acid (~70 g/l) TS, heat to boiling and slowly add to the boiling solution 10 ml of barium chloride (100 g/l) TS. Cover the beaker and heat in a water-bath for 1 hour. Filter, and wash the precipitate several times with small quantities of hot water. Dry and ignite the residue at 600 °C to constant mass. Each g of residue is equivalent to 0.412 g of sulfates (SO_4), calculated with reference to the dried substance; 0.16–0.24 g/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is not more opalescent than opalescence standard TS2 and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry at 105 °C for 3 hours; it loses not more than 0.050 g/g.

Light absorbance. Dissolve 0.050 g in 5 ml of water and measure the absorbance of a 1-cm layer at a wavelength between 260 nm and 280 nm; not greater than 0.1.

Nitrogen. Proceed as described under 2.10 Determination of nitrogen, Method B, using 10 mg of Protamine sulfate; the content of nitrogen is not less than 0.23 g/g and not more than 0.27 g/g, calculated with reference to the dried substance.

Assay. Prepare the following solutions: for solution (A) dissolve 15.0 mg of Protamine sulfate in sufficient water to produce 100 ml; for solution (B) dilute 2.0 ml of solution A to 3.0 ml with water; for solution (C) dilute 1.0 ml of solution A to 3.0 ml with water.

As titrant use a solution of heparin RS in water containing about 170 IU/ml. Titrate each of solutions A, B, and C in duplicate and carry out 3 independent assays. Measure accurately 1.5 ml of one of the solutions and introduce it to a cell of a suitable spectrophotometer set at 420 nm. Add small volumes of the titrant until a sharp change in transmittance is observed and note the volume of titrant added.

For each individual titration, calculate the number of International Units of heparin in the volume of titrant added, per mg of Protamine sulfate. Average the 18 values and test the linearity of the response using the usual statistical methods. The assay is not valid unless the relative standard deviations calculated for the results obtained with each solution are less than 5% of the average result.

Additional requirements for Protamine sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 7.0 IU of endotoxin RS per mg.

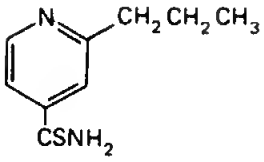
PROTIONAMIDUM

PROTIONAMIDE

Molecular formula. $C_9H_{12}N_2S$

Relative molecular mass. 180.3

Graphic formula.



Chemical name. 2-Propylthioisonicotinamide; 2-propyl-4-pyridinecarbothioamide; CAS Reg. No. 14222-60-7.

Description. Yellow crystals or a crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS and methanol R; slightly soluble in ether R.

Category. Antileprosy drug.

Storage. Protionamide should be kept in a well-closed container, protected from light.

Requirements

Definition. Protionamide contains not less than 98.0% and not more than 101.0% of $C_9H_{12}N_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from protionamide RS or with the *reference spectrum* of protionamide.

B. The absorption spectrum of a 10 µg/ml solution in ethanol (~750 g/l) TS, when observed between 230 nm and 350 nm, exhibits a maximum at about 291 nm; the absorbance of a 1-cm layer at this wavelength is about 0.39.

C. Heat 0.1 g with 5 ml of hydrochloric acid (1 mol/l) VS; the vapours evolved blacken lead acetate paper R.

D. Melting temperature, about 141 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Dissolve 2.0 g in 20 ml of warm methanol R, add 20 ml of water, cool, shake until crystallization occurs, and titrate with sodium hydroxide (0.1 mol/l) VS, using cresol red/ethanol TS as indicator; not more than 0.2 ml is required to obtain the midpoint of the indicator (orange).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. After removing the plate from the chromatographic chamber allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

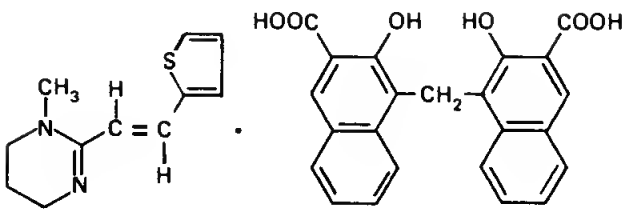
Assay. Dissolve about 0.45 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 18.03 mg of $C_9H_{12}N_2S$.

PYRANTELI EMBONAS
PYRANTEL EMBONATE

Molecular formula. $C_{11}H_{14}N_2S,C_{23}H_{16}O_6$

Relative molecular mass. 594.7

Graphic formula.



Chemical name. (*E*)-1,4,5,6-Tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl]pyrimidine compound with 4,4′-methylenebis[3-hydroxy-2-naphthoate] (1:1); (*E*)-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl]pyrimidine 4,4′-methylenebis[3-hydroxy-2-naphthalenecarboxylate] (1:1); CAS Reg. No. 22204-24-6.

Other name. Pyrantel pamoate.

Description. A yellow, crystalline powder.

Solubility. Practically insoluble in water and methanol R; soluble in dimethyl sulfoxide R; slightly soluble in dimethylformamide R.

Category. Anthelmintic drug.

Storage. Pyrantel embonate should be kept in a well-closed container, protected from light.

Requirements

Definition. Pyrantel embonate contains not less than 97.0% and not more than 103.0% of $C_{11}H_{14}N_2S,C_{23}H_{16}O_6$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrantel embonate RS or with the *reference spectrum* of pyrantel embonate.

B. The absorption spectrum of a 13 µg/ml solution in methanol R, when observed between 230 nm and 360 nm, exhibits 2 maxima at about 288 nm and 300 nm. The ratio of the absorbance at 288 nm to that at 300 nm is about 1.0.

C. Dissolve 5 mg in 1.0 ml of hydrochloric acid (~70 g/l) TS and add 1.0 ml of formaldehyde/sulfuric acid TS; a purple colour is produced.

D. Melting temperature, above 250 °C with decomposition.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 20 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 20 volumes of ethyl acetate R, 5 volumes of methanol R, and 1.5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 100 µl of each of 2 solutions in a mixture of 5 volumes of chloroform R, 5 volumes of methanol R, and 0.5 volumes of ammonia (~260 g/l) TS containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 10 minutes and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

- Perform the assay in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

Transfer about 0.10 g, accurately weighed, to a 200-ml volumetric flask, dissolve in a mixture of 10 ml of dioxan R and 10 ml of ammonia (~100 g/l) TS, and dilute to volume with perchloric acid (~140 g/l) TS. Filter, discard the first 10 ml of the filtrate, and transfer 5 ml of the subsequent filtrate to a 50-ml volumetric flask. Dilute to volume with perchloric acid (~140 g/l) TS and mix. Transfer 25 ml to a 250-ml separating funnel, add 100 ml of chloroform R, and shake well. Drain off the chloroform layer into a second separating funnel. Repeat the extraction of the aqueous phase with a second 100-ml portion of chloroform R, and combine the chloroform extracts into the same separating funnel. Add 40 ml of hydrochloric acid (0.05 mol/l) VS to the combined chloroform extracts and shake well. Drain off the chloroform phase into a third separating funnel and extract with a further 40-ml portion of hydrochloric acid (0.05 mol/l) VS, discarding the chloroform phase. Combine the aqueous phases in a 100-ml volumetric flask, rinse the separating funnel, draining into the volumetric flask, and dilute to volume with hydrochloric acid (0.05 mol/l) VS. Measure the absorbance of a 1-cm layer of this solution at the maximum at about 311 nm against a solvent cell containing hydrochloric acid (0.05 mol/l) VS. Calculate the amount of $C_{11}H_{14}N_2S$,$C_{23}H_{16}O_6$ in the substance being tested by comparison with pyrantel embonate RS, similarly and concurrently examined.

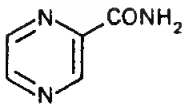
PYRAZINAMIDUM

PYRAZINAMIDE

Molecular formula. $C_5H_5N_3O$

Relative molecular mass. 123.1

Graphic formula.



Chemical name. Pyrazinecarboxamide; CAS Reg. No. 98-96-4.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Sparingly soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Category. Antituberculosis drug.

Storage. Pyrazinamide should be kept in a well-closed container.

Requirements

Definition. Pyrazinamide contains not less than 98.5% and not more than 101.0% of $C_5H_5N_3O$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrazinamide RS or with the *reference spectrum* of pyrazinamide.

B. The absorption spectrum of a 10 µg/ml solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 268 nm and another smaller one at 310 nm; the absorbance of a 1-cm layer at 268 nm is about 0.66.

C. Dissolve 0.1 g in 10 ml of water and add 1 ml of ferrous sulfate (15 g/l) TS; an orange-red colour develops turning to blue on the addition of 1 ml of sodium hydroxide (~80 g/l) TS.

D. Boil 20 mg with 5 ml of sodium hydroxide (~200 g/l) TS; ammonia, perceptible by its odour, is evolved.

Melting range. 188–191 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 1 g of the substance; the water content is not more than 5.0 mg/g.

pH value. pH of a 15 mg/ml solution in carbon-dioxide-free water R, 5.0–7.0.

Ammonia. To 20 ml of carbon-dioxide-free water R add 1.0 ml of formaldehyde TS and 0.05 ml of phenolphthalein/ethanol TS, neutralize with carbonate-free sodium hydroxide (0.1 mol/l) VS, if necessary, add 0.50 g of the test substance, and dissolve cautiously while heating. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS until a pink colour is obtained; not more than 0.50 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is required.

Assay. Dissolve about 0.07 g, accurately weighed, in 15 ml of chloroform R and 5 ml of acetic anhydride R, add 0.15 ml of sudan red TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 12.31 mg of $C_5H_5N_3O$.

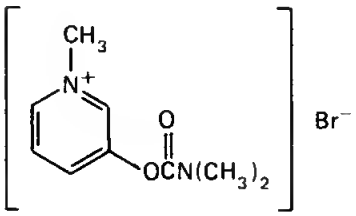
PYRIDOSTIGMINI BROMIDUM

PYRIDOSTIGMINE BROMIDE

Molecular formula. $C_9H_{13}BrN_2O_2$

Relative molecular mass. 261.1

Graphic formula.



Chemical name. 3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate; 3-[[(dimethylamino)carbonyl]oxy]-1-methylpyridinium bromide; CAS Reg. No. 101-26-8.

Description. A white or almost white, crystalline powder; odour, agreeable, characteristic.

Solubility. Soluble in less than 1 part of water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cholinergic.

Storage. Pyridostigmine bromide should be kept in a well-closed container, protected from light.

Additional information. Pyridostigmine bromide is deliquescent.

Requirements

Definition. Pyridostigmine bromide contains not less than 98.5% and not more than 101.0% of $C_9H_{13}BrN_2O_2$, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 25 µg/ml solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 270 nm; the absorbance of a 1-cm layer at this wavelength is about 0.46 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

B. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. To 0.1 g add 0.6 ml of sodium hydroxide (~80 g/l) TS; an orange colour is produced. Warm the solution; the colour changes to yellow and the vapours evolved turn moistened red litmus paper blue.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of bromides.

Melting range. 153–156 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 20 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R1 as the coating substance and a mixture of

67 volumes of water, 30 volumes of methanol R, and 3 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10µl of each of 3 solutions containing (A) 20mg of the test substance per ml, (B) 0.10mg of the test substance per ml, and (C) 0.10mg of pyridostigmine bromide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, spray it with 4-nitroaniline TS2 and then with sodium hydroxide (0.1mol/l) VS. Dry the plate again in a current of warm air, spray it with potassium iodobismuthate TS2 and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5g, accurately weighed, in 30ml of glacial acetic acid R1, add 10ml of mercuric acetate/acetic acid TS and 2 drops of quinaldine red/ethanol TS as indicator, and titrate with perchloric acid (0.1mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1mol/l) VS is equivalent to 26.11mg of $C_9H_{13}BrN_2O_2$.

Additional requirements for Pyridostigmine bromide for parenteral use

Complies with the monograph for "Parenteral preparations".

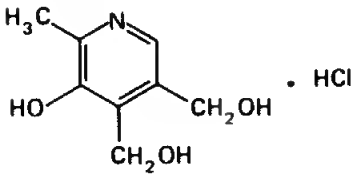
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 17.0IU of endotoxin RS per mg.

PYRIDOXINI HYDROCHLORIDUM
PYRIDOXINE HYDROCHLORIDE

Molecular formula. $C_8H_{11}NO_3,HCl$

Relative molecular mass. 205.6

Graphic formula.



Chemical name. 5-Hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride; 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine hydrochloride; CAS Reg. No. 58-56-0.

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Vitamin.

Storage. Pyridoxine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Pyridoxine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Pyridoxine hydrochloride contains not less than 98.5% and not more than 101.0% of $C_8H_{11}NO_3$,HCl, calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 10 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 290 nm; the absorbance of a 1-cm layer at this wavelength is about 0.43 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

B. The absorption spectrum of a 0.5 mg/ml solution in phosphate buffer pH 6.9, TS, when observed between 230 nm and 350 nm, exhibits maxima at about 254 nm and 324 nm; the absorbances of a 1-cm layer at the maximum wavelengths are about 0.18 and 0.35, respectively (preferably use 2-cm cells for the measurement and calculate the absorbances of 1-cm layers).

C. In each of two test-tubes A and B, place 1 ml of a 0.1 mg/ml solution and 2 ml of sodium acetate (150 g/l) TS. To tube A add 1 ml of water and to tube B 1 ml of boric acid (50 g/l) TS and mix. Cool both tubes to about 20 °C and rapidly add to each tube 1 ml of 2,6-dichloroquinone chlorimide/ethanol TS; a blue colour is produced in tube A, whereas in tube B no blue colour is observed.

D. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 0.5 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 40 µg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R; it loses not more than 5.0 mg/g.

pH value. pH of a 10 mg/ml solution, 2.3–3.5.

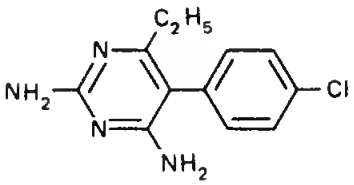
Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.56 mg of $C_8H_{11}NO_3$,HCl.

PYRIMETHAMINUM
PYRIMETHAMINE

Molecular formula. $C_{12}H_{13}ClN_4$

Relative molecular mass. 248.7

Graphic formula.



Chemical name. 2,4-Diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine; 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine; CAS Reg. No. 58-14-0.

Description. A white, crystalline powder; odourless.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antimalarial.

Storage. Pyrimethamine should be kept in a well-closed container, protected from light.

Requirements

Definition. Pyrimethamine contains not less than 99.0% and not more than 101.0% of $C_{12}H_{13}ClN_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained with pyrimethamine RS or with the *reference spectrum* of pyrimethamine.

B. The absorption spectrum of a 15 µg/ml solution in hydrochloric acid (0.005 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 272 nm and a minimum at about 260 nm; the absorbance of a 1-cm layer at the maximum is about 0.48 and at the minimum about 0.45.

C. To 0.5 g add 1 ml of a mixture of equal volumes of glacial acetic acid R and acetic anhydride R and heat under a reflux condenser for 30 minutes. Pour the warm mixture into 25 ml of water; a white, crystalline precipitate is produced. Collect the precipitate on a filter, wash with water, recrystallize from 4 ml of ethanol (~750 g/l) TS mixed with 6 ml of water, and dry at 105 °C; melting temperature, about 172 °C (2,4-diacetylpyrimethamine).

D. Ignite 0.1 g with 0.5 g of anhydrous sodium carbonate R, extract the residue with water, and filter. Neutralize with nitric acid (~130 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Melting range. 239–242 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity or alkalinity. Boil 0.3 g with 15 ml of water, cool and filter. Add 0.25 ml of methyl red/ethanol TS to the filtrate; a yellow colour is observed. Not more than 0.1 ml of hydrochloric acid (0.05 mol/l) VS is required to change the colour of the solution to red.

Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.20 ml of quinaldine red/ethanol TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration,

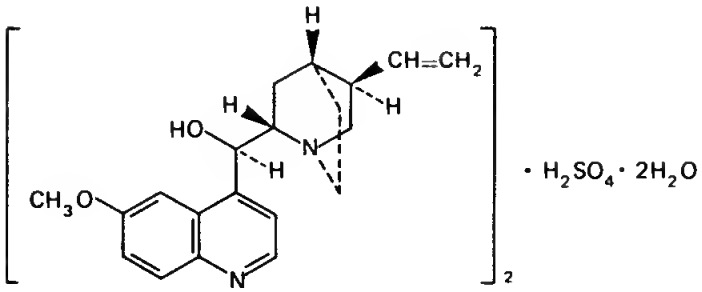
Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.87 mg of $C_{12}H_{13}ClN_4$.

QUINIDINI SULFAS
QUINIDINE SULFATE

Molecular formula. $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

Relative molecular mass. 783.0

Graphic formula.



Chemical name. Quinidine sulfate (2:1) (salt), dihydrate; (9*S*)-6′-methoxycinchonan-9-ol sulfate (2:1) (salt), monohydrate; CAS Reg. No. 6591-63-5 (dihydrate).

Description. Colourless, needle-like crystals or a white, crystalline powder; odourless.

Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R and acetone R.

Category. Antidysrhythmic drug.

Storage. Quinidine sulfate should be kept in a well-closed container, protected from light.

Additional information. Quinidine sulfate has a very bitter taste. It darkens in colour on exposure to light.

Requirements

Definition. Quinidine sulfate contains not less than 99.0% and not more than 101.0% of total alkaloids, calculated as $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$ and with reference to the dried substance.

Identity tests

A. Dissolve 0.10 g in 10 ml of water; the solution produces a slight blue fluorescence (keep the remaining solution for test B). To 1.0 ml add a few drops of sulfuric acid (~100 g/l) TS, and dilute with water to 5 ml; a vivid blue fluorescence is produced.

B. To 1.0 ml of the solution prepared for test A, add 4 ml of water, 0.15 ml of bromine TS1, and 1.0 ml of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. Dissolve 0.05 g in 5 ml of hot water, cool, add 1 ml of silver nitrate (40 g/l) TS, and stir with a glass rod; after a few minutes a white precipitate is produced, which is soluble in nitric acid (~130 g/l) TS.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 20 mg/ml solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +275$ to $+290°$.

Clarity and colour of solution. Dissolve 0.20 g in 10 ml of hydrochloric acid (0.1 mol/l) VS; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 130 °C; it loses not less than 30 mg/g and not more than 50 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 6.0–6.8.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 20 volumes of toluene R, 12 volumes of ether R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 4 µl of each of 4 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 0.25 mg of quinine R per ml, (C) 0.25 mg of cinchonine R per ml, and (D) 10 mg of the test substance dissolved in 1 ml of solution C. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 15 minutes and repeat the development. Heat the plate at 105 °C for 30 minutes, allow it to cool, spray it with potassium iodoplatinate TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B or solution C. Disregard any spot obtained with solution A immediately

below the principal spot. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots.

Dihydroquinidine. Dissolve about 0.2 g, accurately weighed, in 20 ml of water. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS, and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes. Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow colour. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 18.67 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$, calculated with reference to the dried substance. Express the results of both the above determination and the assay in percentages. The difference between the two is not more than 15%.

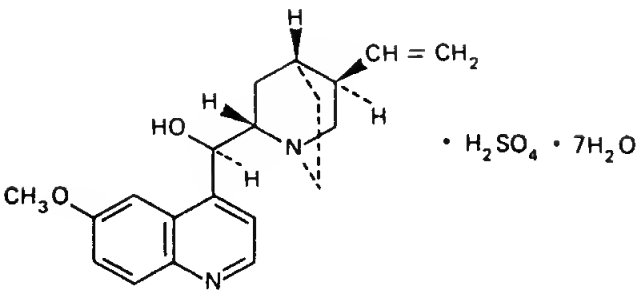
Assay. Dissolve about 0.20 g, accurately weighed, in 10 ml of chloroform R and 20 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.90 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

QUININI BISULFAS
QUININE BISULFATE

Molecular formula. $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$

Relative molecular mass. 548.6

Graphic formula.



Chemical name. Quinine sulfate (1:1) (salt), heptahydrate; (8α*S*,9*R*)-6′-methoxycinchonan-9-ol sulfate (1:1) (salt), heptahydrate; (8*S*,9*R*)-9-hydroxy-

6′-methoxycinchonan sulfate (1 : 1) (salt), heptahydrate; CAS Reg. No. 6183-68-2 (heptahydrate).

Description. Colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antimalarial drug.

Storage. Quinine bisulfate should be kept in a well-closed container, protected from light.

Additional information. Quinine bisulfate effloresces in dry air. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Quinine bisulfate contains not less than 98.5% and not more than 101.5% of total alkaloids, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$ and with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 10 ml of water and add 0.05 ml of sulfuric acid (~100 g/l) TS; a strong blue fluorescence is produced (keep the solution for test B).

B. To the solution prepared for test A add 0.15 ml of bromine TS1 and 1.0 ml of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. Dissolve 0.05 g in 5 ml of water and add 1 ml of silver nitrate (40 g/l) TS; no white precipitate is produced.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 30 mg/ml solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = –208 to –216°.

Clarity and colour of solution. Dissolve 0.20 g in 10 ml of hydrochloric acid (0.1 mol/l) VS; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 18 hours; it loses not less than 190 mg/g and not more than 240 mg/g.

pH value. pH of a 10 mg/ml solution, 2.8–3.4.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 20 volumes of toluene R, 12 volumes of ether R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 4 µl of each of 4 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 0.25 mg of quinine R per ml, (C) 0.25 mg of cinchonidine R per ml, and (D) 10 mg of the test substance dissolved in 1 ml of solution C. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 15 minutes and repeat the development. Heat the plate at 105 °C for 30 minutes, allow it to cool, spray it with potassium iodoplatinate TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B or solution C. Disregard any spot obtained with solution A immediately below the principal spot. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots.

Dihydroquinine. Dissolve about 0.2 g, accurately weighed, in 20 ml of water. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS, and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes. Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow colour. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 21.13 mg of $C_{20}H_{24}N_2O_2,H_2SO_4$, calculated with reference to the dried substance. Express the results of both the above determination and the assay in percentages. The difference between the two is not more than 10%.

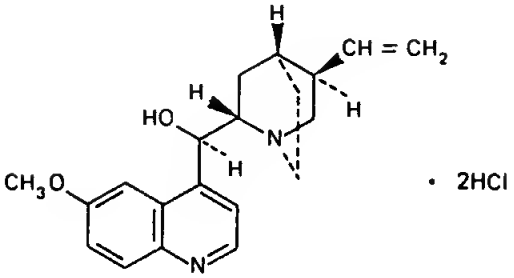
Assay. Dissolve about 0.45 g, accurately weighed, in 15 ml of water. Add 25 ml of sodium hydroxide (0.1 mol/l) VS and extract with 3 quantities, each of 25 ml of chloroform R. Wash the combined chloroform extracts with 20 ml of water. Dry the chloroform extracts with anhydrous sodium sulfate R, evaporate to dryness under reduced pressure, and dissolve the residue in 50 ml of glacial acetic acid R1. Titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 21.13 mg of $C_{20}H_{24}N_2O_2,H_2SO_4$.

QUININI DIHYDROCHLORIDUM
QUININE DIHYDROCHLORIDE

Molecular formula. $C_{20}H_{24}N_2O_2,2HCl$

Relative molecular mass. 397.3

Graphic formula.



Chemical name. (8α*S*,9*R*)-6′-Methoxycinchonan-9-ol dihydrochloride; (8*S*, 9*R*)-9-hydroxy-6′-methoxycinchonan dihydrochloride; CAS Reg. No. 60-93-5.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Very soluble in water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antimalarial drug.

Storage. Quinine dihydrochloride should be kept in a well-closed container, protected from light.

Additional information. Quinine dihydrochloride turns yellow on exposure to light. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Quinine dihydrochloride contains not less than 99.0% and not more than 101.0% of total alkaloids, calculated as $C_{20}H_{24}N_2O_2,2HCl$ and with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 10 ml of water and add 0.05 ml of sulfuric acid (~100 g/l) TS; a strong blue fluorescence is produced (keep the solution for test B).

B. To the solution prepared for test A add 0.15 ml of bromine TS1 and 1.0 ml of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. Dissolve 0.05 g in 5 ml of water and add 1 ml of silver nitrate (40 g/l) TS; a white precipitate is produced.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 30 mg/ml solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = –223 to –229°.

Clarity and colour of solution. Dissolve 0.20 g in 10 ml of hydrochloric acid (0.1 mol/l) VS; the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 30 mg/g.

pH value. pH of a 30 mg/ml solution, 2.0–3.0.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 20 volumes of toluene R, 12 volumes of ether R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 4 µl of each of 4 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 0.25 mg of quinine R per ml, (C) 0.25 mg of cinchonidine R per ml, and (D) 10 mg of the test substance dissolved in 1 ml of solution C. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 15 minutes and repeat the development. Heat the plate at 105 °C for 30 minutes, allow to cool, spray with potassium iodoplatinate TS and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B or solution C. Disregard any spot obtained with solution A immediately below the principal spot. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots.

Dihydroquinine. Dissolve about 0.2 g, accurately weighed, in 20 ml of water. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS, and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) TS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes.

Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow colour. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2$,2HCl, calculated with reference to the dried substance. Express the results of both the above determination and the assay in percentages. The difference between the two is not more than 10%.

Assay. Dissolve about 0.3 g, accurately weighed, in 50 ml of glacial acetic acid R1, add 20 ml of acetic anhydride R and 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2$,2HCl.

Additional requirement for Quinine dihydrochloride for parenteral use

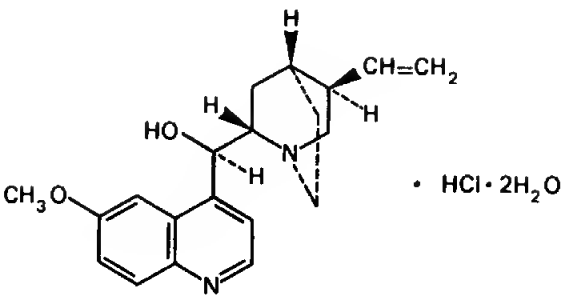
Complies with the monograph for "Parenteral preparations".

QUININI HYDROCHLORIDUM
QUININE HYDROCHLORIDE

Molecular formula. $C_{20}H_{24}N_2O_2$,HCl,2H_2O

Relative molecular mass. 396.9

Graphic formula.



Chemical name. (8α,9*R*)-6′-Methoxycinchonan-9-ol monohydrochloride (salt) dihydrate; (8α,9*R*)-9-hydroxy-6′-methoxycinchonan hydrochloride (1:1) (salt) dihydrate; CAS Reg. No. 6119-47-7.

Description. Silky, colourless crystals, often grouped in clusters; odourless.

Solubility. Soluble in water; freely soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

Category. Antimalarial.

Storage. Quinine hydrochloride should be kept in a well-closed container, protected from light.

Additional information. Quinine hydrochloride has a very bitter taste.

Requirements

Definition. Quinine hydrochloride contains not less than 98.5% and not more than 101.0% of total alkaloids, calculated as $C_{20}H_{24}N_2O_2$,HCl and with reference to the dried substance.

Identity tests

A. Dissolve 0.1 g in 2.5 ml of water; the solution is not fluorescent. Dilute 0.5 ml of this solution to 100 ml with water and add 2 drops of sulfuric acid (~100 g/l) TS; a strong blue fluorescence is produced.

B. To 5 ml of a 1 mg/ml solution add 2–3 drops of bromine TS1 and 5 drops of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. A 10 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 20 mg/ml solution in hydrochloric acid (0.1 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = –240 to –258 °.

Barium. To 15 ml of a 0.3 g/ml solution add 1 ml of sulfuric acid (~100 g/l) TS; the solution remains clear for not less than 15 minutes.

Sulfates. Dissolve 0.5 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Clarity and colour of solution. A solution of 0.10 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 60 mg/g and not more than 100 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 6.0–7.0.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 20 volumes of toluene R, 12 volumes of ether R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 4 µl of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per ml and (B) 0.25 mg of cinchonidine R per ml. After removing the plate from the chromatographic chamber, heat it at 105 °C for 30 minutes, allow it to cool, spray with potassium iodoplatinate TS, and examine the plate in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Dihydroquinine. Dissolve about 0.2 g, accurately weighed, in 20 ml of water. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes. Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow coloration. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 18.04 mg of $C_{20}H_{24}N_2O_2$,HCl. Express the results of both the above determination and the assay in percentages, calculated with reference to the dried substance. The difference between the two is not more than 10%.

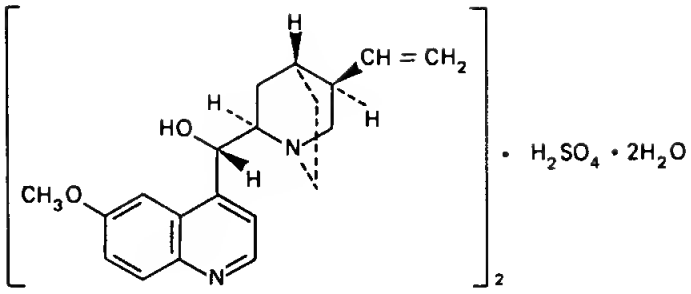
Assay. Dissolve about 0.35 g, accurately weighed, in 50 ml of glacial acetic acid R1, add 20 ml of acetic anhydride R and 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 18.04 mg of $C_{20}H_{24}N_2O_2$,HCl.

QUININI SULFAS
QUININE SULFATE

Molecular formula. $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$

Relative molecular mass. 783.0

Graphic formula.



Chemical name. (8α,9*R*)-6′-Methoxycinchonan-9-ol sulfate (2:1) (salt) dihydrate; (8α,9*R*)-9-hydroxy-6′-methoxycinchonan sulfate (2:1) (salt) dihydrate; CAS Reg. No. 6591-63-5.

Description. Colourless, needle-like crystals; odourless.

Solubility. Slightly soluble in water, ethanol (~750 g/l) TS, and ether R.

Category. Antimalarial.

Storage. Quinine sulfate should be kept in a well-closed container, protected from light.

Additional information. Quinine sulfate has a very bitter taste.

Requirements

Definition. Quinine sulfate contains not less than 99.0% and not more than 101.0% of total alkaloids, calculated as $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$ and with reference to the dried substance.

Identity tests

A. Dissolve 5 mg in 10 ml of water and add 1 drop of sulfuric acid (~100 g/l) TS; a strong blue fluorescence is produced.

B. To 5 ml of a 1 mg/ml solution add 2–3 drops of bromine TS1 and 5 drops of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 30 mg/ml solution in sulfuric acid (~100 g/l) TS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -240$ to $-250\,°$.

Clarity and colour of solution. Dissolve 20 mg in 5 ml of hydrochloric acid (0.1 mol/l) VS and add sufficient water to produce 10 ml. This solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 30 mg/g and not more than 50 mg/g.

pH value. pH of a 10 mg/ml suspension in carbon-dioxide-free water R, 5.7–6.6.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 20 volumes of toluene R, 12 volumes of ether R, and 5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 4 µl of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per ml and (B) 0.25 mg of cinchonidine R per ml. After removing the plate from the chromatographic chamber, heat it at 105 °C for 30 minutes, allow it to cool, spray with potassium iodoplatinate TS and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Dihydroquinine. Dissolve about 0.2 g, accurately weighed, in 20 ml of water. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes. Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow coloration. Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 24.90 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$. Express the results of both the above determination and the assay in percentages, calculated with reference to the dried substance. The difference between the two is not more than 10%.

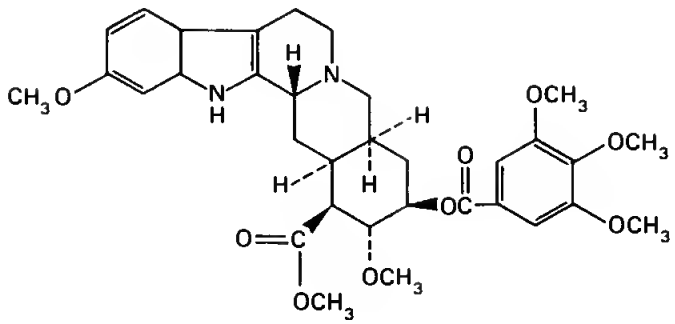
Assay. Dissolve about 0.20 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 20 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 24.90 mg of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

RESERPINUM
RESERPINE

Molecular formula. $C_{33}H_{40}N_2O_9$

Relative molecular mass. 608.7

Graphic formula.



Chemical name. Methyl 18β-hydroxy-11,17α-dimethoxy-3β,20α-yohimban-16β-carboxylate 3,4,5-trimethoxybenzoate (ester); methyl 11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylate; CAS Reg. No. 50-55-5.

Description. Small, white to pale beige crystals or a white to pale beige, crystalline powder; odourless.

Solubility. Practically insoluble in water; soluble in 90 parts of acetone R; very slightly soluble in methanol R, ethanol (~750 g/l) TS, and ether R.

Category. Neuroleptic; hypotensive.

Storage. Reserpine should be kept in a well-closed container, protected from light.

Additional information. Reserpine darkens slowly on exposure to light, but more rapidly in solution.

Requirements

Definition. Reserpine contains not less than 98.0% and not more than 102.0% of $C_{33}H_{40}N_2O_9$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from reserpine RS or with the *reference spectrum* of reserpine.

B. To 1 mg add 0.2 ml of a freshly prepared 10 g/l solution of vanillin R in hydrochloric acid (~250 g/l) TS; a pink colour is produced in about 2 minutes.

C. Mix 0.5 mg with 5 mg of 4-dimethylaminobenzaldehyde R and 0.2 ml of glacial acetic acid R and add 0.2 ml of sulfuric acid (~1760 g/l) TS; a green colour is produced. Add 1 ml of glacial acetic acid R; the colour changes to red.

Specific optical rotation. Use a 10 mg/ml solution in chloroform R; $[\alpha]_D^{20\,°C}$ = −113 to −127 °.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 10 mg/g.

Oxidation products. Measure the absorbance of a 1-cm layer of a 0.2 mg/ml solution in glacial acetic acid R at the maximum at about 388 nm; not greater than 0.10 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Assay

- The solutions must be protected from air and light throughout the assay.

Moisten about 25 mg, accurately weighed, with 2 ml of ethanol (~750 g/l) TS, add 2 ml of sulfuric acid (0.25 mol/l) VS and 10 ml of ethanol (~750 g/l) TS, and warm gently to effect solution. Cool, dilute to 100.0 ml with ethanol (~750 g/l) TS, and dilute 5.0 ml of this solution to 50.0 ml with the same solvent. Transfer 10.0 ml of the solution to a boiling tube, add 2.0 ml of sulfuric acid (0.25 mol/l) VS and 2.0 ml of freshly prepared sodium nitrite (3 g/l) TS, mix, and heat in a water-bath at 55 °C for 30 minutes. Cool, add 1.0 ml of freshly prepared sulfamic acid (50 g/l) TS, and dilute to 25.0 ml with ethanol (~750 g/l) TS. Measure the absorbance of a 1-cm layer at the maximum at about 390 nm, against a solvent cell containing a solution prepared by treating a further 10.0 ml of the solution in the same manner but omitting the sodium nitrite. Calculate the amount of $C_{33}H_{40}N_2O_9$ in the substance being tested by comparison with reserpine RS, similarly and concurrently examined. In an adequately cal-

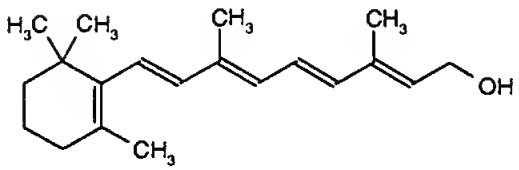
ibrated spectrophotometer the absorbance of the reference solution should be 0.42 ± 0.01 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Additional requirements for Reserpine for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 71.5 IU of endotoxin RS per mg.

RETINOLUM DENSATUM OLEOSUM
RETINOL CONCENTRATE, OILY FORM



Chemical name. 3,7 Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol; CAS Reg. No. 68-26-8.

Other name. Vitamin A concentrate (oily form).

Description. A yellow to brownish yellow, oily liquid.

Solubility. Practically insoluble in water; soluble or partly soluble in dehydrated ethanol R; miscible with organic solvents.

Category. Vitamin.

Storage. The oily form of Retinol concentrate should be kept in a well-closed and well-filled container, protected from light, and stored at a temperature between 8 and 15 °C. Once the container has been opened its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

Labelling. The designation on the container should state the name of the ester or esters, whether any additional agents are added and their quantities, as well as the method of solubilizing the liquid if partial crystallization has occurred.

Additional information. Even in the absence of light, the oily form of Retinol concentrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Partial crystallization may occur in concentrated solutions and upon refrigeration.

Requirements

Definition. The oily form of Retinol concentrate consists of an ester or a mixture of esters (acetate, propionate, or palmitate) of retinol ($C_{20}H_{30}O$), usually prepared by synthesis. It may be diluted in a suitable vegetable oil. It may contain suitable antimicrobial agents and stabilizing agents such as antioxidants.

The declared content of retinol is not less than **500 000 IU/g.** Retinol concentrate contains not less than **95.0%** and not more than **110.0%** of the amount of $C_{20}H_{30}O$ stated on the label.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 8 volumes of cyclohexane R and 2 volumes of ether R as the mobile phase. Apply separately to the plate 2 µl of each of 4 solutions in cyclohexane R containing (A) 2 mg of Retinol per ml, (B) 2 mg of retinol acetate RS per ml, (C) 2 mg of retinol propionate RS per ml, and (D) 2 mg of retinol palmitate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with antimony trichloride TS. Examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds to one or more of the spots obtained with solutions B, C, and D.

B. Dissolve a small drop in about 1 ml of dichloromethane R and add 5 ml of antimony trichloride TS; a blue colour is immediately produced which turns gradually to violet-red.

Acid value. Not more than 2.0.

Peroxides. For solution (A) dissolve 0.30 g in 25 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. For solution (B) prepare a solution containing 0.27 g of ferric chloride R per ml, and add 1.0 ml to 99 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. Dilute 2.0 ml to 100 ml with the same solvent mixture.

Place in 2 separate test-tubes in the following order, mixing after each addition, 3 ml of a solution containing 18 mg of ammonium thiocyanate R per ml, 10 ml of methanol R, 0.3 ml of ferrous sulfate/hydrochloric acid TS, and 15 ml of toluene R. Then add 1.0 ml of solution A into one tube and 1.0 ml of solution B into the other, shake, and allow to stand for 5 minutes. The colour produced with solution A is not more intense than that produced with solution B.

Assay

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and oxidizing agents, and maintaining whenever possible an atmosphere of nitrogen above the solution.

The spectrophotometric measurements should be made at 20–25 °C. Before each series of measurements, check the wavelength scale of the spectrophotometer as well as the absorbance scale. The cells filled with 2-propanol R must not differ from each other in absorbance by more than 0.002 at each of the following wavelengths: 300 nm, 325 nm, 350 nm, and 370 nm.

Carry out each determination in duplicate, using separately weighed amounts of Retinol concentrate. Prepare a dilution series containing 25–100 mg of Retinol concentrate in 5 ml of *n*-pentane R and dilute with 2-propanol R to a presumed concentration of 10–15 IU per ml. Verify that the absorption maximum of the solution to be examined, measured against a solvent cell containing 2-propanol R, lies between 325 nm and 327 nm. Measure the absorbances at 300 nm, 326 nm, 350 nm, and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_γ/A_{326} for each wavelength. If the ratios do not exceed 0.592 at 300 nm, 0.537 at 350 nm, and 0.142 at 370 nm, calculate the content of retinol in International Units per gram from the expression: $A_{326} \times V \times 1900/100\,m$, where A_{326} is the absorbance at 326 nm, V is the total volume used for the dilution to give 10–15 IU per ml, m is the mass of Retinol concentrate in g, and 1900 is the factor to convert the specific absorbances of ester of retinol into IU per g.

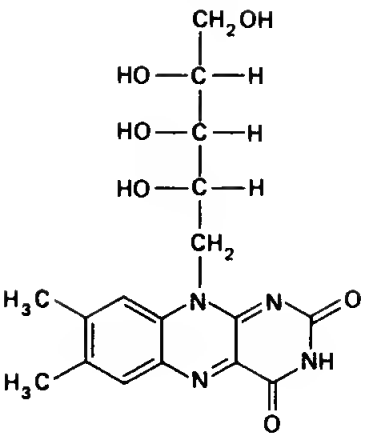
RIBOFLAVINUM

RIBOFLAVIN

Molecular formula. $C_{17}H_{20}N_4O_6$

Relative molecular mass. 376.4

Graphic formula.



Chemical name. 7,8-Dimethyl-10-(D-*ribo*-2,3,4,5-tetrahydroxypentyl)isoalloxazine; CAS Reg. No. 83-88-5.

Description. A yellow to orange-yellow, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; practically insoluble in ethanol (~750 g/l) TS, ether R, and acetone R.

Category. Vitamin.

Storage. Riboflavin should be kept in a tightly closed container, protected from light.

Additional information. Riboflavin has a bitter taste. Solutions of Riboflavin, especially in dilute solutions of alkalis, deteriorate rapidly when exposed to light.

Requirements

Definition. Riboflavin contains not less than 98.0% and not more than 102.0% of $C_{17}H_{20}N_4O_6$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from riboflavin RS or with the *reference spectrum* of riboflavin.

B. Dissolve 1 mg in 100 ml of water. The solution has a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence

by reflected light. The addition of mineral acids or alkalis destroys the fluorescence.

Sulfated ash. Not more than 3.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 15 mg/g.

Lumiflavin. Shake 25 mg with 10 ml of ethanol-free chloroform R for 5 minutes and filter. Measure the absorbance of the filtrate in a 1-cm layer at the maximum at about 440 nm against a solvent cell containing ethanol-free chloroform R; the absorbance does not exceed 0.025 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Assay

- Carry out the operations in subdued light.

To about 0.075 g, accurately weighed, add 5 ml of water to ensure that the substance is completely wetted. Then add 5 ml of sodium hydroxide (~80 g/l) TS. As soon as this is completely dissolved, add 100 ml of water and 2.5 ml of glacial acetic acid R, and sufficient water to produce 1000 ml. To 10 ml of this solution, add 1 ml of sodium acetate (50 g/l) TS and sufficient water to produce 50 ml. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 444 nm. Calculate the amount of $C_{17}H_{20}N_4O_6$ in the substance being tested by comparison with riboflavin RS, similarly and concurrently examined.

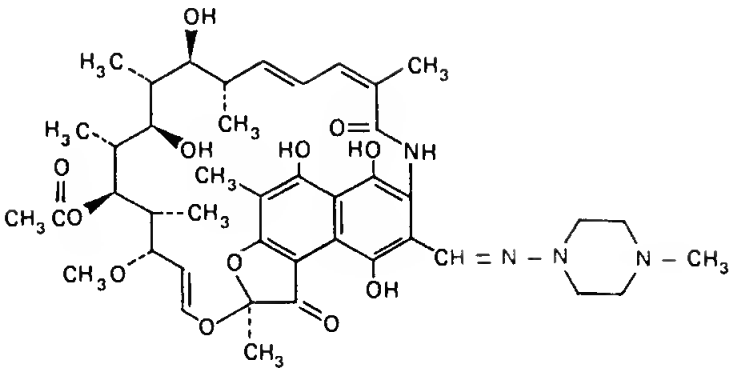
RIFAMPICINUM

RIFAMPICIN

Molecular formula. $C_{43}H_{58}N_4O_{12}$

Relative molecular mass. 823.0

Graphic formula.



Chemical name. (2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*S*,23*S*,24*E*)-5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca[1,11,13]-trienimino)naphtho[2,1-*b*]-furan-1,11-(2*H*-dione, 21-acetate; 3-[[(4-methyl-1-piperazinyl)imino]methyl]rifamycin; CAS Reg. No. 13292-46-1.

Other name. Rifampin.

Description. A brick red to red-brown, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; freely soluble in chloroform R; soluble in methanol R; slightly soluble in acetone R, ethanol (~750 g/l) TS, and ether R.

Category. Antileprosy drug; antituberculosis drug.

Storage. Rifampicin should be kept in a tightly closed container, protected from light and stored at a temperature not exceeding 15 °C, or in an atmosphere of nitrogen at a temperature not exceeding 30 °C.

Requirements

Definition. Rifampicin contains not less than 97.0% and not more than 102.0% of $C_{43}H_{58}N_4O_{12}$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from rifampicin RS or with the *reference spectrum* of rifampicin.

B. Dissolve 50 mg in 50 ml of methanol R and dilute 1 ml of this solution to 50 ml with phosphate buffer, pH 7.4, TS. The absorption spectrum of the resulting solution, when observed between 220 nm and 500 nm, exhibits 4 maxima at about 237 nm, 254 nm, 334 nm, and 475 nm; the ratio of the absorbance of a 1-cm layer at the maximum at about 334 nm to that at the maximum at about 475 nm is about 1.75.

C. Suspend 25 mg in 25 ml of water, shake for 5 minutes and filter. To 5 ml of the nitrate add 1 ml of ammonium persulfate/phosphate buffer TS and shake for a few minutes; the colour turns from orange-yellow to violet-red without the formation of a precipitate.

Heavy metals. Place 1.0 g in a silica crucible and mix it with 4 ml of magnesium sulfate/sulfuric acid TS. Heat cautiously to ignition and continue heating until a white or at most greyish residue is obtained. Ignite at a temperature not exceeding 800 °C, allow to cool, and moisten the residue with a few drops of sulfuric acid (~100 g/l) TS. Evaporate, ignite again, and allow to cool. Next, dissolve the residue in hydrochloric acid (~70 g/l) TS, add, drop by drop, a solution of ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, also drop by drop, acetic acid (~60 g/l) PbTS to adjust the pH to 3–4, filter, dilute with water to 40 ml, and mix. Determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 10 mg/g.

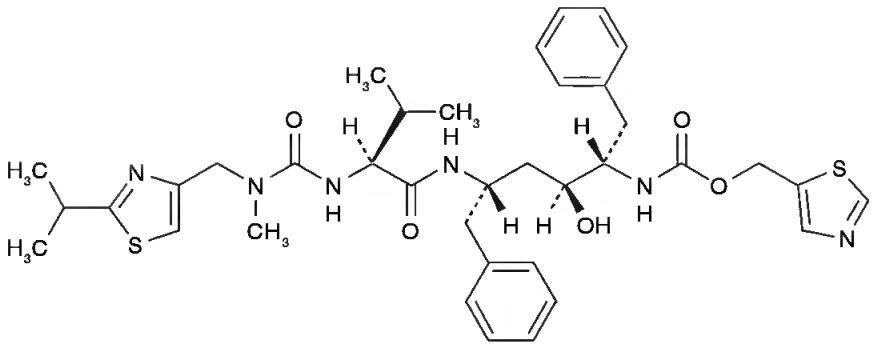
pH value. Shake 0.10 g with 10 ml of carbon-dioxide-free water R; pH of the suspension, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and preparing the slurry with phosphate/citrate buffer pH 6.0, TS. As the mobile phase use a mixture of 85 volumes of chloroform R and 15 volumes of methanol R. Apply separately to the plate 20 µl of each of 4 solutions in chloroform R containing (A) 20 mg of the test substance per ml, (B) 0.10 mg of 3-formylrifamycin SV RS per ml, (C) 0.30 mg of rifampicin quinone RS per ml, and (D) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in daylight. Any coloured spots obtained with solution A, other than the principal spot, are not more intense than the corresponding spots obtained with solutions B and C. Any other spots obtained with solution A are not more intense than that obtained with solution D.

Assay. Dissolve about 0.10 g, accurately weighed, in sufficient methanol R to produce 100 ml. Dilute 2 ml of this solution to 100 ml with phosphate buffer, pH 7.4, TS. Measure the absorbance of the resulting solution in a 1-cm layer at the maximum at about 475 nm, using as the blank phosphate buffer, pH 7.4, TS. Calculate the content of $C_{43}H_{58}N_4O_{12}$, using the absorptivity value of 18.7 ($A^{1\%}_{1\,cm}$ = 187).

RITONAVIRUM

RITONAVIR



$C_{37}H_{48}N_6O_5S_2$

Relative molecular mass. 721.0

Chemical name. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl] carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate; CAS Reg. NO.155213-67-5.

Description. A white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methanol R, sparingly soluble in acetone R and very slightly soluble in acetonitrile R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Ritonavir should be kept in a well-closed container, protected from light.

Additional information. Ritonavir may exhibit polymorphism.

Requirements

Ritonavir contains not less than **98.5%** and not more than **101.0%** of $C_{37}H_{48}N_6O_5S_2$, calculated with reference to the dried substance.

Identity tests

- Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of ritonavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of ritonavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with basic potassium permanganate (5 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 40 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 240 nm; the specific absorbance ($A^{1\%}_{1\,cm}$) is 116 to 128.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ritonavir RS or with the *reference spectrum* of ritonavir. If the spectra obtained in the solid-state show differences, dissolve the test substance and the reference substance separately in a minimal amount of methanol R, crystallise by adding just enough water drop by drop, filter and dry for about one hour and record the spectra again.

Specific optical rotation. Use a 20.0 mg/ml solution in methanol R; $[\alpha]_D^{20\,°C}$ = +7 ° to +10 °.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 2 hours at 105 °C; it loses not more than 5 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 35 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 37 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 2 volumes of purified water.

Prepare the sodium phosphate buffer pH 4.0 by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate R and 1.88 g of sodium hexanesulfonate R in 800 ml of purified water, adjust the pH to 4.0 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–20	70	30	Isocratic
20–30	70 to 0	30 to 100	Linear gradient
30–40	0	100	Isocratic
40–45	0 to 70	100 to 30	Linear gradient
45–50	70	30	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 µg of ritonavir per ml.

For the system suitability test: prepare solution (3) using 5 ml of solution (1) and 1 ml of sulfuric acid (475 g/l), heat in a boiling water bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 240 nm.

Maintain the column temperature at 35 °C.

Inject 20 µl of solution (3). The test is not valid unless the resolution between the principal peak (retention time about 22 minutes) and the peak with a relative retention of about 0.8 is not less than 3.5. The test is also not valid unless the resolution between the principal peak and the peak with a relative retention of about 1.5 is not less than 9.0. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 20 µl each of solutions (1) and (2).

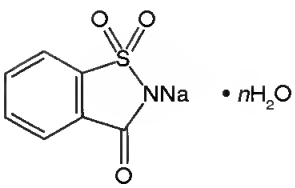
In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than three times the area of the principal peak obtained with solution (2) (0.3%). In the chromatogram obtained with solution (1), the areas of not more than two peaks, other than the principal peak, are greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the areas of not more than four such peaks are greater than the area of the principal peak obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than ten times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under 2.6 Non-aqueous titration Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.05 mg of $C_{37}H_{48}N_6O_5S_2$.

SACCHARINUM NATRICUM

SACCHARIN SODIUM

Saccharin sodium, anhydrous
Saccharin sodium, dihydrate



$n = 0$ (anhydrous)
$n = 2$ (dihydrate)

$C_7H_4NNaO_3S$ (anhydrous)
$C_7H_4NNaO_3S,2H_2O$ (dihydrate)

Relative molecular mass. 205.2 (anhydrous); 241.2 (dihydrate).

Chemical name. 1,2-Benzisothiazolin-3-one 1,1-dioxide, sodium salt; 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, sodium salt; CAS Reg. No. 128-44-9 (anhydrous).

1,2-Benzisothiazolin-3-one 1,1-dioxide, sodium salt, dihydrate; 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, sodium salt, dihydrate; CAS Reg. No. 6155-57-3 (dihydrate).

Other name. Saccharimidum natricum.

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Sweetening agent.

Storage. Saccharin sodium should be kept in a well-closed container.

Additional information. Saccharin sodium effloresces slowly in air and loses about half of its content of water of crystallization. It has a very sweet taste, even in very dilute solutions.

Requirements

Saccharin sodium contains not less than **98.0%** and not more than the equivalent of **101.0%** of $C_7H_4NNaO_3S$, calculated with reference to the anhydrous substance.

Identity tests

A. To 20 mg add 0.04 g of resorcinol R and 0.5 ml of sulfuric acid (~1760 g/l) TS, and heat gently until a dark green colour is observed. Allow to cool and add 10 ml of water and 10 ml of sodium hydroxide (~80 g/l) TS; a fluorescent green solution is produced.

B. Ignite 1 g and proceed with the residue as follows:

— Dissolve half of the residue in acetic acid (~60 g/l) TS. When tested for sodium as described under 2.1 General identification tests it yields reaction B.

— Dissolve the remaining residue in hydrochloric acid (~70 g/l) TS. It yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Arsenic. Transfer 3.3 g to a crucible containing 3.3 g of anhydrous sodium carbonate R. Moisten with a small quantity of water, evaporate to dryness on a water-bath, and ignite to 550 °C until all black particles have disappeared. Cool, dissolve the residue in 5 ml of hydrochloric acid (~250 g/l) AsTS, and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 1 g of Saccharin sodium dihydrate; the water content is not more than 150 mg/g.

Free acid or alkali. Dissolve 1 g in 10 ml of carbon-dioxide-free water R, add 5 ml of sulfuric acid (0.005 mol/l) VS, boil, cool, and titrate with sodium hydroxide (0.01 mol/l) VS using phenolphthalein/ethanol TS as indicator; 4.5–5.5 ml are required to obtain a pink colour.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 100 volumes of chloroform R, 50 volumes of methanol R, and 10 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of the following four solutions: For solution (A) dissolve 2.6 g of Saccharin sodium in 10 ml of sodium hydrogen carbonate (100 g/l) TS, add 12.5 g of diatomaceous support R as a filter-aid, and mix well. Transfer to a chromatographic tube, 250 mm in length and with a diameter of 25 mm, fitted at the lower end with a sintered-glass disc and a stopcock. Pack the contents of the tube by tapping on a padded surface and tamping firmly from the top. Elute with dichloromethane R at a rate of 50 ml in 30 minutes. Evaporate the eluate to dryness and dissolve the residue in 4 ml of acetone R. For solutions (B) dissolve 50 µg of toluene-2-sulfonamide RS per ml of acetone R, (C) 5 mg of Saccharin sodium per ml of methanol R, and (D) 50 µg of 4-sulfamoylbenzoic acid R per ml of acetone R. After removing the plate from the chromatographic chamber, dry in a current of warm air, heat at 105 °C for 5 minutes, and spray the hot plate with sodium hypochlorite TS1. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a faint

blue colour with 0.05 ml of a solution of 5 mg of potassium iodide R in 1 ml of starch TS containing 1 % glacial acetic acid R. Avoid prolonged exposure to cold air. Spray the plate again with the same mixture. Examine the chromatogram in daylight.

Any spot obtained with solution A corresponding to toluene-2-sulfonamide is not more intense than that obtained with solution B. Any spot obtained with solution C corresponding to 4-sulfamoylbenzoic acid is not more intense than that obtained with solution D.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

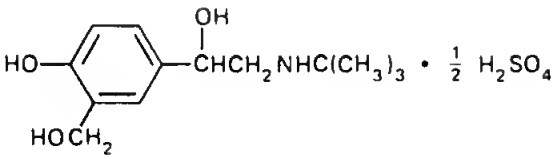
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

SALBUTAMOLI SULFAS
SALBUTAMOL SULFATE

Molecular formula. $(C_{13}H_{21}NO_3)_2,H_2SO_4$

Relative molecular mass. 288.4

Graphic formula.



Chemical name. α^1-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α, α'-diol sulfate (2:1) (salt); α^1-[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol sulfate (2:1) (salt); CAS Reg. No. 51022-70-9.

Description. A white or almost white powder; odourless.

Solubility. Soluble in 4 parts of water; slightly soluble in ethanol (~750 g/l) TS, and ether R.

Category. Antiasthmatic drug.

Storage. Salbutamol sulfate should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Salbutamol sulfate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Salbutamol sulfate contains not less than 98.0% and not more than 101.0% of $C_{13}H_{21}NO_3,{}^1/_2H_2SO_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from salbutamol sulfate RS or with the *reference spectrum* of salbutamol sulfate.

B. The absorption spectrum of a 0.080 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 276 nm; the absorbance of a 1-cm layer at this wavelength is about 0.46.

C. Dissolve 0.05 g in 5 ml of water and add 0.1 ml of ferric chloride (25 g/l) TS; a reddish violet colour is produced. Add 0.05 g of sodium hydrogen carbonate R; a fleshy precipitate is produced with an evolution of gas. Add a few drops of sulfuric acid (~1760 g/l) TS; the solution becomes colourless.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 4 volumes of ammonia (~260 g/l) TS, 16 volumes of water, 30 volumes of 2-propanol R, and 50 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 20 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing

the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Place the plate for a few minutes in an atmosphere saturated with diethylamine R, spray it with diazotized sulfanilic acid TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.9 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 57.67 mg of $C_{13}H_{21}NO_3,{}^1/_2H_2SO_4$.

Additional requirement for Salbutamol sulfate for parenteral use

Complies with the monograph for "Parenteral preparations".

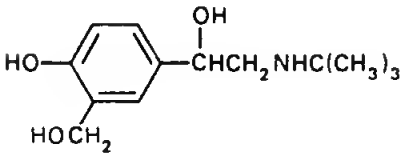
SALBUTAMOLUM

SALBUTAMOL

Molecular formula. $C_{13}H_{21}NO_3$

Relative molecular mass. 239.3

Graphic formula.



Chemical name. α^1-[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene-α, α′-diol; α^1-[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol; CAS Reg. No. 18559-94-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Soluble in 70 parts of water; soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Antiasthmatic drug.

Storage. Salbutamol should be kept in a well-closed container, protected from light.

Requirements

Definition. Salbutamol contains not less than 98.0% and not more than 101.0% of $C_{13}H_{21}NO_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from salbutamol RS or with the *reference spectrum* of salbutamol.

B. The absorption spectrum of a 0.080 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum only at about 276 nm; the absorbance of a 1-cm layer at this wavelength is about 0.56.

C. Dissolve 0.05 g in 5 ml of water and add 0.1 ml of ferric chloride (25 g/l) TS; a reddish violet colour is produced. Add 0.05 g of sodium hydrogen carbonate R; a fleshy precipitate is produced with an evolution of gas. Add a few drops of sulfuric acid (~1760 g/l) TS; the solution becomes colourless.

D. Melting temperature, about 155 °C with decomposition.

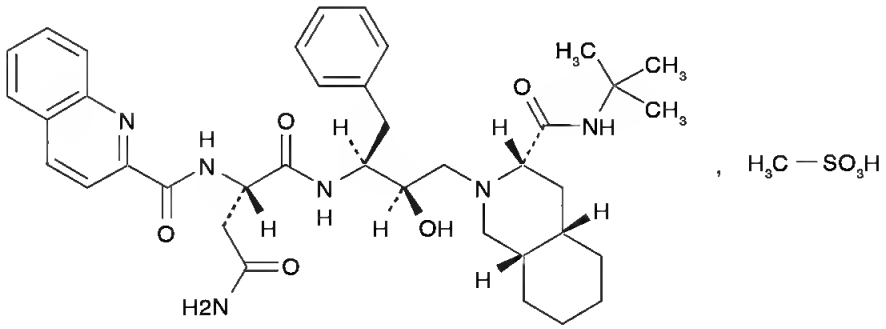
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 50 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 4 volumes of ammonia (~260 g/l) TS, 16 volumes of water, 30 volumes of 2-propanol R, and 50 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 20 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Place the plate for a few minutes in an atmosphere saturated with diethylamine R, spray it with diazotized sulfanilic acid TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.4 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 23.93 mg of $C_{13}H_{21}NO_3$.

SAQUINAVIRI MESILAS
SAQUINAVIR MESILATE



$C_{38}H_{50}N_6O_5.CH_4O_3S$

Relative molecular mass. *767.0*

Chemical name. (2*S*)-*N*^{1}-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl) carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide methanesulfonate; CAS Reg. No. 149845-06-7.

Description. A white or almost white powder.

Solubility. Very slightly soluble in water and sparingly soluble in methanol R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Saquinavir mesilate should be kept in a tightly-closed container, protected from light.

Additional information. Saquinavir mesilate is slightly hygroscopic.

Requirements

Definition. Saquinavir mesilate contains not less than **98.5%** and not more than **101.0%** of $C_{38}H_{50}N_6O_5.CH_4O_3S$ calculated with reference to the dried substance.

Manufacture. The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Identity tests

- Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions in methanol R (A) 5 mg of the test substance per ml and (B) 5 mg of saquinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions in methanol R (A) 5 mg of the test substance per ml and (B) 5 mg of saquinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in basic potassium permanganate (~1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 239 nm; the specific absorbance ($A^{1\%}_{1\,cm}$) is 580 to 640.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from saquinavir mesilate RS or with the *reference spectrum* of saquinavir mesilate.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -33$ to $-39°$.

Heavy metals. Use 0.5 g in 30 ml of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts of methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate R in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Comments
0–25	100	0	Isocratic
25–45	100 to 45	0 to 55	Linear gradient
45–55	45	55	Isocratic
55–60	45 to 100	55 to 0	Linear gradient
60–70	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 µg per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 30 °C.

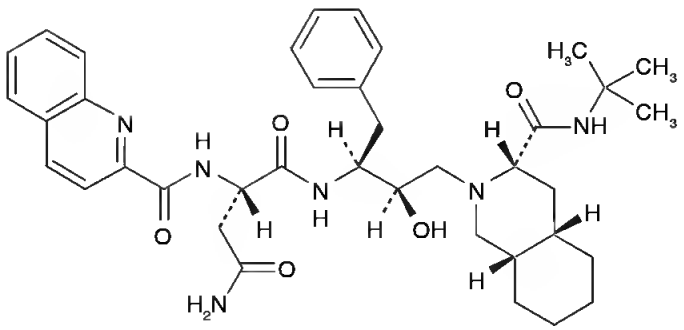
Inject 20 µl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time about 21 minutes) and the peak of similar size with a relative retention of about 0.45 is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 2.0. The relative retention of these two peaks is about 1.8 and 1.9, respectively. If necessary, adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the area of not more than one such peak is greater than the area of the principal peak obtained with solution 2 (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve about 0.500 g, accurately weighed, in 70 ml of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS determining the end-point potentiometrically. Perform a blank determination and make the necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 76.70 mg of $C_{38}H_{50}N_6O_5.CH_4O_3S$; calculate with reference to the dried substance.

SAQUINAVIRUM
SAQUINAVIR



$C_{38}H_{50}N_6O_5$

Relative molecular mass. 670.8

Chemical name. (2*S*)-*N*'-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl) carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide; CAS Reg. NO.127779-20-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol.

Category. Antiretroviral (Protease Inhibitor).

Storage. Saquinavir should be kept at 2–8 °C in a tightly closed container, protected from light.

Additional information. Saquinavir is slightly hygroscopic.

Requirements

Saquinavir contains not less than **98.5%** and not more than **101.0%** of $C_{38}H_{50}N_6O_5$, calculated with reference to the dried substance.

Identity tests

- Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 8

volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 5 mg of the test substance per ml and (B) 5 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 5 mg of the test substance per ml and (B) 5 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in dilute basic potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 20 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 238 nm; the specific absorbance ($A^{1\%}_{1\,cm}$) is 670 to 730.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from saquinavir RS or with the *reference spectrum* of saquinavir.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R; $[\alpha]_D^{20\,°C}$ = –50° to –56°.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 20 mg/g.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm ×

4.6mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5µm).

Use the following conditions for gradient elution:

Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88g of anhydrous sodium dihydrogen phosphate R in 800ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (~105g/l) TS and dilute to 1000ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–25	100	0	Isocratic
25–45	100 to 45	0 to 55	Linear gradient
45–55	45	55	Isocratic
55–60	45 to 100	55 to 0	Linear gradient
60–70	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5µg of saquinavir per ml.

For the system suitability test: prepare solution (3) using 2ml of solution (1) and 5ml of sulfuric acid (475g/l), heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220nm.

Maintain the column temperature at 30°C.

Inject 20µl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time about 21 minutes) and the peak of similar size with a relative retention of about 0.45 is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 2.0. The relative retention of these two peaks is about 1.8 and

1.9, respectively. If necessary, adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the area of not more than one such peak is greater than the area of the principal peak obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.300 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under 2.6 Non-aqueous titration method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.54 mg of $C_{38}H_{50}N_6O_5$; calculate with reference to the dried substance.

SELENII DISULFIDUM
SELENIUM DISULFIDE

SeS_2

Relative molecular mass. 143.1

Chemical name. Selenium sulfide; CAS Reg. No. 7488-56-4.

Description. A bright orange to reddish brown powder.

Solubility. Selenium disulfide is practically insoluble in water and organic solvents.

Category. Antifungal drug.

Storage. Selenium disulfide should be kept in a well-closed container.

Requirements

Selenium disulfide contains not less than **52.0%** and not more than **55.5%** of Se.

Identity tests

A. Gently boil 0.05 g with 5 ml of nitric acid (~1000 g/l) TS for 30 minutes, dilute to 50 ml with water, and filter. To 5 ml of the filtrate add 10 ml of water and 5 g of urea R, boil, cool, and add 2.0 ml of potassium iodide (80 g/l) TS; a yellow to orange colour is produced which darkens rapidly on standing. (Keep this solution for test B.)

B. Allow the coloured solution obtained in test A to stand for 10 minutes, and filter through kieselguhr R1. The filtrate yields the reactions described under 2.1 General identification tests as characteristic of sulfates.

Sulfated ash. Not more than 2.0 mg/g.

Soluble selenium compounds. For solution A, use 10 g of Selenium disulfide, add 100 ml of water, mix well, allow to stand for 1 hour with frequent shaking, and filter. For solution B, use a solution of selenious acid R containing 5 µg of selenium per ml. To 10 ml of each of solutions A and B, add 2 ml of a solution containing about 1 ml of formic acid (~1080 g/l) TS in 10 ml of water, and dilute both solutions to 50 ml with water. If necessary, adjust the pH to 2.5 ± 0.5 with the diluted formic acid as prepared above. Then add 2.0 ml of freshly prepared 3,3′-diaminobenzidine tetrahydrochloride (5 g/l) TS, allow to stand for 45 minutes, and adjust the pH to 6.5 ± 0.5 with ammonia (~100 g/l) TS. Shake both solutions for 1 minute with 10 ml of toluene R, and allow to separate. Measure the absorbances of a 1-cm layer of the toluene layers at 420 nm against a solvent cell containing the same reagents treated as described above. The absorbance of solution A is not more than that of solution B (5 µg of Se per g).

Assay. To about 0.1 g, accurately weighed, add 25 ml of fuming nitric acid R, heat on a water-bath for 1 hour, cool, and dilute to 100 ml with water. To 25 ml of this solution add 50 ml of water and 5 g of urea R, and heat to boiling. Cool, add 7 ml of potassium iodide (80 g/l) TS, 3 ml of starch TS, and titrate immediately with sodium thiosulfate (0.1 mol/l) VS. Repeat the procedure without the Selenium disulfide being examined and make any necessary corrections.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 1.974 mg of Se.

SENNAE FOLIUM

SENNA LEAF

Alexandrian Senna leaf
Tinnevelly Senna leaf

Description. Odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Category. Cathartic drug.

Storage. Senna leaf should be kept protected from light and moisture.

Additional information. Even in the absence of light, Senna leaf is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Senna leaf consists of the dried leaflets of *Cassia senna* L., known as Alexandrian or Khartoum Senna (*C. acutifolia* Delile) or Tinnevelly Senna (*C. angustifolia* Vahl), or a mixture of both species.

Senna leaf contains not less than 2.5% of hydroxyanthracene derivatives, calculated as sennoside B.

Identity test

To about 0.5 g of the powdered leaf add 10 ml of sodium hydroxide/ethanol TS and boil on a water-bath, dilute with 10 ml of water, and filter. Acidify the filtrate with hydrochloric acid (~70 g/l) TS and extract with 10 ml of ether R. Separate the ether layer and shake it with 5 ml of ammonia (~100 g/l) TS; a yellowish red colour is produced in the ammonia layer.

Macroscopic examination

Alexandrian Senna leaf. Pale greyish green, thin, fragile leaflets; lanceolate, mucronate; length, 20–40 mm; width, 5–15 mm, the maximum width being at a point slightly below the centre; lamina, slightly undulant; both surfaces covered with fine, short trichomes; pinnate venation, slightly prominent midrib with lateral veins leaving the midrib at an angle of about 60° and anastomosing to form a ridge parallel to the margin.

Tinnevelly Senna leaf. Yellowish green leaflets; elongated and lanceolate; length, 25–50 mm; width at the centre, 7–20 mm; lamina, flat; both surfaces are smooth with a very small number of trichomes, and marked with impressed transverse or oblique lines.

Microscopic examination. Epidermis with polygonal cells containing mucilage; unicellular thick-walled trichomes, length, up to 260 µm, slightly curved at the base, warty; paracytic stomata on both surfaces; under the epidermal cells a single row of palisade layer; cluster crystals of calcium oxalate distributed throughout the lacunose tissue; on the adaxial surface of the leaf, sclerenchymatous fibres and a gutter-shaped group of similar fibres on the abaxial side containing prismatic crystals of calcium oxalate.

Water-soluble extractive. Shake 5 g with a mixture of 0.5 ml of chloroform R and 200 ml of water, filter, evaporate 20 ml of the filtrate and weigh; the residue is not less than 300 mg/g.

Acid-insoluble ash. Carry out the procedure as described under 4.1 Determination of ash and acid-insoluble ash; not more than 20 mg/g.

Stems and foreign matter. Weigh about 200 g and spread it in a thin layer. Detect the stems and the foreign matter by eye or with the use of a 6× lens; separate and weigh individually the stems and the foreign matter; not more than 20 mg/g of stems and not more than 10 mg/g of foreign matter.

Assay. Place 0.15 g of the powdered leaf in a 100-ml flask. Add 30.0 ml of water, mix, weigh and place in a water-bath at 80–90 °C. Heat under a reflux condenser for 15 minutes. Allow to cool, weigh, and adjust to the original mass with water. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separating funnel. Add 0.1 ml of hydrochloric acid (~70 g/l) TS and shake with 3 quantities, each of 15 ml, of chloroform R. Allow to separate and discard the chloroform layer. Add 0.10 g of sodium hydrogen carbonate R and shake for 3 minutes. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100-ml round-bottomed flask with a ground-glass neck. Add 20 ml of ferric chloride (65 g/l) TS and mix. Heat for 20 minutes under a reflux condenser in a water-bath with the water level above that of the liquid in the flask; add 1 ml of hydrochloric acid (~420 g/l) TS and heat for a further 20 minutes, with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of ether R previously used to rinse the flask. Combine the ether layers and wash with 2 quantities, each of 15 ml, of water. Transfer the ether layers to a volumetric flask and dilute to 100 ml with ether R. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a solution containing 5 mg of magnesium acetate R per ml of methanol R. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 515 nm against a solvent cell containing methanol R. Calculate in % the amount of hydroxyanthracene derivatives as sennoside B with an absorptivity value of 24.0 ($A^{1\%}_{1\,cm} = 240$) as follows: $1.25\,A/W$, where A is the absorbance at 515 nm and W is the mass of the material examined in g.

SENNAE FRUCTUS

SENNA FRUIT

Alexandrian Senna fruit
Tinnevelly Senna fruit

Description. Odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Category. Cathartic drug.

Storage. Senna fruit should be kept protected from light and moisture.

Additional information. Even in the absence of light, Senna fruit is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Alexandrian Senna fruit is the dried ripe fruit of *Cassia senna* L., (*C. acutifolia* Delile) and Tinnevelly Senna fruit is the dried ripe fruit of *Cassia angustifolia* Vahl.

Alexandrian Senna fruit contains not less than 3.4% of hydroxyanthracene derivatives and Tinnevelly Senna fruit contains not less than 2.2% of hydroxyanthracene derivatives, both calculated as sennoside B.

Identity test
Cut the fruit into small pieces or powder about 0.5 g, add 10 ml of sodium hydroxide/ethanol TS and boil on a water-bath, dilute with 10 ml of water and filter. Acidify the filtrate with hydrochloric acid (~70 g/l) TS and extract with 10 ml of ether R. Separate the ether layer and shake it with 5 ml of ammonia (~100 g/l) TS; a pink to red colour is produced in the ammonia layer.

Macroscopic examination. Leaflike, flat and thin pods, yellowish green to yellowish brown with a dark brown central area, oblong or reniform.

Alexandrian Senna fruit. Pale to greyish green; length, about 40–50 mm; width, 20–25 mm; stylar point at one end, containing 6–7 obovate green to pale brown seeds, with longitudinal prominent ridges on the testa.

Tinnevelly Senna fruit. Brown to greyish black; length, about 35–60 mm; width, 14–18 mm; stylar point at one end, containing up to 10 obovate green to pale brown seeds, with indefinite transverse ridges.

Microscopic examination. Epicarp with very thick cuticularized isodiametrical cells, occasional *anomocytic* or *paracytic* stomata, and very few unicellular and warty trichomes; hypodermis with collenchymatous cells; mesocarp with parenchymatous tissue containing a layer of calcium oxalate prisms; endocarp consisting of thick-walled fibre, mostly perpendicular to the longitudinal axis of the fruit, but the inner fibres running at an oblique angle or parallel to the longitudinal axis. Seeds, subepidermal layer of palisade cells with thick outer walls; the endosperm has polyhedral cells with mucilaginous walls.

Water-soluble extractive. Shake 5 g with a mixture of 0.5 ml of chloroform R and 200 ml of water, filter, evaporate 20 ml of the filtrate and weigh; the residue is not less than 250 mg/g.

Acid-insoluble ash. Carry out the procedure as described under 4.1 Determination of ash and acid-insoluble ash; not more than 20 mg/g.

Foreign matter. Weigh about 200 g and spread it in a thin layer. Detect the foreign matter by eye or with the use of a 6× lens, separate and weigh; not more than 10 mg/g.

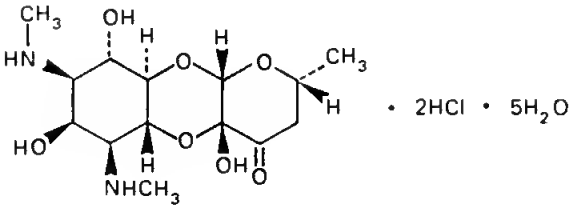
Assay. Place 0.15 g of the powdered fruit in a 100-ml flask. Add 30.0 ml of water, mix, weigh, and place in a water-bath at 80–90 °C. Heat under a reflux condenser for 15 minutes. Allow to cool, weigh, and adjust to the original mass with water. Centrifuge and transfer 20.0 ml of the supernatant liquid to a 150-ml separating funnel. Add 0.1 ml of hydrochloric acid (~70 g/l) TS and shake with 3 quantities, each of 15 ml, of chloroform R. Allow to separate and discard the chloroform layer. Add 0.10 g of sodium hydrogen carbonate R and shake for 3 minutes. Centrifuge and transfer 10.0 ml of the supernatant liquid to a 100-ml round-bottomed flask with a ground-glass neck. Add 20 ml of ferric chloride (65 g/l) TS and mix. Heat for 20 minutes under a reflux condenser in a water-bath with the water level above that of the liquid in the flask; add 1 ml of hydrochloric acid (λ420 g/l) TS and heat for a further 20 minutes, with frequent shaking, to dissolve the precipitate. Cool, transfer the mixture to a separating funnel and shake with 3 quantities, each of 25 ml, of ether R previously used to rinse the flask. Combine the ether layers and wash with 2 quantities, each of 15 ml, of water. Transfer the ether layers to a volumetric flask and dilute to 100 ml with ether R. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a solution containing 5 mg of magnesium acetate R per ml of methanol R. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 515 nm against a solvent cell containing methanol R. Calculate in % the amount of hydroxyanthracene derivatives as sennoside B with an absorptivity value of 24.0 ($A^{1\%}_{1\,cm}$ = 240) as follows: $1.25A/W$, where A is the absorbance at 515 nm and W is the mass of the material examined in g.

SPECTINOMYCINI HYDROCHLORIDUM
SPECTINOMYCIN HYDROCHLORIDE

Molecular formula. $C_{14}H_{24}N_2O_7,2HCl,5H_2O$

Relative molecular mass. 495.4

Graphic formula.



Chemical name. (2*R*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-Decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate; [2*R*-(2α,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ)]-decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4*H*-pyrano[2,3-*b*][1,4] benzodioxin-4-one dihydrochloride pentahydrate; CAS Reg. No. 22189-32-8 (pentahydrate).

Description. A white or almost white, crystalline powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS, chloroform R and ether R.

Category. Antibacterial drug.

Storage. Spectinomycin hydrochloride should be kept in a well-closed container.

Requirements

Definition. Spectinomycin hydrochloride contains not less than 600 µg of $C_{14}H_{24}N_2O_7$ per mg, calculated with reference to the anhydrous substance.

Manufacture. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine-like substances. Carry out the test as described under 3.6 Test for histamine-like substances (vasodepressor substances), using 1 ml per kg of body mass of a solution in saline TS containing 25 mg of the substance to be examined per ml.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from Spectinomycin hydrochloride RS or with the *reference spectrum* of Spectinomycin hydrochloride.

B. A 20 mg/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 0.10 g/ml solution, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +15$ to $+21\,°$.

Sulfated ash. Not more than 10 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 0.16 g/g and not more than 0.20 g/g.

pH value. pH of a 0.10 g/ml solution, 3.8–5.6.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 10 volumes of 1-propanol R, 8 volumes of water, 1 volume of glacial acetic acid R, and 1 volume of pyridine R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions containing (A) 20 mg of the test substance per ml and (B) 0.20 ml of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it with potassium permanganate (25 g/l) TS, allow it to stand for 2–3 minutes, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure.

Assay. Carry out the assay described under 1.14.5 Gas chromatography. As an internal standard use a solution containing 2 mg of triphenylantimony R per ml of dimethylformamide R. Use the following 2 solutions: (1) to about 30 mg of spectinomycin hydrochloride RS, accurately weighed, add 10.0 ml of the internal standard and 1.0 ml of hexamethyldisilazane R, and shake intermittently for 1 hour; and (2) to 30 mg of the substance being examined add 10.0 ml of the internal standard and 1.0 ml of hexamethyldisilazane R, and shake intermittently for 1 hour. For the procedure use a flame ionization detector and a glass column 1.3 m long and 0.4 cm in internal diameter packed with an adequate quantity of an adsorbent composed of 5 g of phenyl/methylpolysiloxane R supported on 95 g of acid-washed and base-washed, silanized kieselguhr R4. Maintain the column and the detector at about 215 °C and 270 °C, respectively,

and the injection part at about 265 °C. Use dry helium R as the carrier gas at a flow rate of about 90 ml per minute. Prepare chromatogram A and B injecting separately about 2.5 µl of each of solutions 1 and 2. Measure the area of the major peak in each chromatogram, and calculate the content in µg of $C_{14}H_{24}N_2O_7$ per mg in the substance being tested.

Additional requirement for Spectinomycin hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

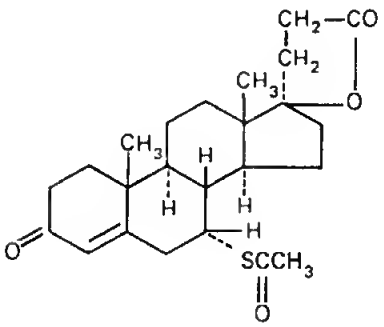
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.09 IU of endotoxin RS per mg of spectinomycin.

SPIRONOLACTONUM
SPIRONOLACTONE

Molecular formula. $C_{24}H_{32}O_4S$

Relative molecular mass. 416.6

Graphic formula.



Chemical name. 17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate; 7α-(acetylthio)-17-hydroxy-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone; CAS Reg. No. 52-01-7.

Description. A light yellowish white to light yellowish brown powder; odourless or with a faint characteristic odour.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS.

Category. Diuretic.

Storage. Spironolactone should be kept in a well-closed container, protected from light.

Additional information. Spironolactone may show preliminary melting at about 135 °C, followed by resolidification.

Requirements

Definition. Spironolactone contains not less than 97.0% and not more than 101.5% of $C_{24}H_{32}O_4S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from Spironolactone RS or with the *reference spectrum* of Spironolactone.

B. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 230 nm and 350 nm, exhibits a maximum at about 238 nm; the absorbance of a 1-cm layer at this wavelength is about 0.47.

C. Shake 10 mg with 2 ml of sulfuric acid (~700 g/l) TS; an orange solution with an intense yellowish green fluorescence is produced. Heat the solution gently; the colour changes to deep red and hydrogen sulfide, which blackens lead acetate paper R, is evolved. Pour the solution into water; a greenish yellow, opalescent solution is produced.

D. Melting temperature, about 204 °C with decomposition.

Specific optical rotation. Use a 10 mg/ml solution in chloroform R: $[\alpha]_D^{20\,°C}$ = –33.0 to –37.0 °.

Chromium. Place 0.20 g with 1 g of potassium carbonate R and 0.3 g of potassium nitrate R into a platinum crucible; mix, heat gently until fused, and ignite at 600–650 °C until the carbon is removed. Cool, dissolve the residue as completely as possible in 10 ml of water using gentle heat, filter, and dilute with sufficient water to produce 20 ml. To 10 ml add 0.5 g of urea R and acidify with sulfuric acid (~190 g/l) TS, dilute to 20 ml with water, and add 0.5 ml of diphenylcarbazide TS. The colour produced is not deeper than that of a solution obtained by adding 1 ml of sulfuric acid (~190 g/l) TS to 0.50 ml of a freshly prepared solution containing 2.83 mg of potassium dichromate R in 100 ml of water, then diluting to 20 ml with water and adding 0.5 ml of diphenylcarbazide TS.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance (a precoated plate from a commercial source is suitable) and butyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in chloroform R containing (A) 20 mg of the test substance per ml and (B) 0.20 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry at room temperature and develop the plate a second time allowing the solvent to ascend 15 cm above the line of application. Remove the plate, allow the solvent to evaporate at room temperature, spray it with sulfuric acid/methanol TS, and heat at 105 °C for 10 minutes. Examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Mercapto compounds. Shake 2.0 g with 20 ml of water, filter, and titrate 10 ml of the filtrate with iodine (0.005 mol/l) VS, using starch TS as indicator. Repeat the operation without the test substance and make any necessary corrections. Not more than 0.1 ml of iodine (0.005 mol/l) VS is required.

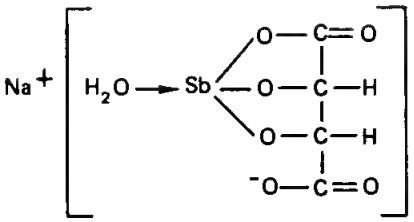
Assay. Dissolve about 10 mg, accurately weighed, in sufficient methanol R to produce 100 ml and dilute 10 ml of this solution to 100 ml with methanol R. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 238 nm, and calculate the content of $C_{24}H_{32}O_4S$, using the absorptivity value of 47 ($A^{1\%}_{1\,cm} = 470$).

STIBII NATRII TARTRAS
ANTIMONY SODIUM TARTRATE

Molecular formula. $C_4H_4NaO_7Sb$

Relative molecular mass. 308.8

Graphic formula.



Chemical name. Sodium aqua[tartrato(4⁻)-O^1,O^2,O^3]antimoniate(III); CAS Reg. No. 34521-09-0.

Description. Colourless, transparent scales or an almost white powder; odourless.

Solubility. Soluble in 1.5 parts of water; practically insoluble in ethanol (~750 g/l) TS.

Category. Antischistosomal drug.

Storage. Antimony sodium tartrate should be kept in a tightly closed container, protected from light.

Additional information. Antimony sodium tartrate is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Antimony sodium tartrate contains not less than 98.0% and not more than 101.0% of $C_4H_4NaO_7Sb$, calculated with reference to the dried substance.

Identity tests

A. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 10 mg/ml solution.

B. Dissolve 0.05 g in 1.0 ml of hydrochloric acid (~70 g/l) TS and add 1.0 ml of hydrogen sulfide TS; an orange-red precipitate is produced, which is soluble in ammonium sulfide TS and in sodium hydroxide (~80 g/l) TS.

C. Dissolve 20 mg in 0.2 ml of water; it yields reaction B described under 2.1 General identification tests, as characteristic of tartrates.

Arsenic. Dissolve 1.3 g in 10 ml of water and add 16 ml of stannated hydrochloric acid (~250 g/l) AsTS in a flask, connect to a condenser, and distil 20 ml; wash the flask and condenser, return the distillate and washings to the flask, add 0.05 ml of stannous chloride AsTS, and redistil 16 ml; proceed with the distillate as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 8 µg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of water is clear and colourless.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 60 mg/g.

Acidity or alkalinity. Dissolve 1.0 g in 50 ml of water and add a few drops of bromocresol green/ethanol TS; not more than 2 ml of either hydrochloric acid (0.01 mol/l) VS or sodium hydroxide (0.01 mol/l) VS are required to obtain the midpoint of the indicator (green) indicative of pH 4.5.

Assay. Dissolve about 0.5 g, accurately weighed, in 50 ml of water, add 5 g of potassium sodium tartrate R and 2 g of sodium tetraborate R. Titrate with iodine (0.05 mol/l) VS using starch TS as indicator, added towards the end of the titration. Each ml of iodine (0.05 mol/l) VS is equivalent to 15.44 mg of $C_4H_4NaO_7Sb$.

STREPTOMYCINI SULFAS

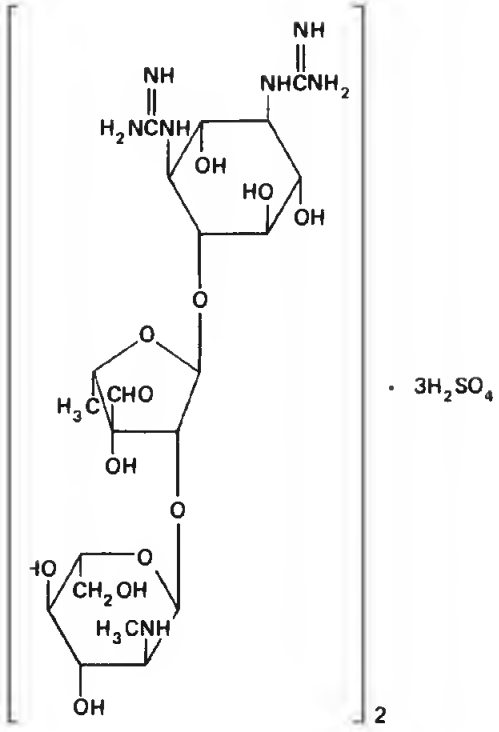
STREPTOMYCIN SULFATE

Streptomycin sulfate (non-injectable)
Streptomycin sulfate, sterile

Molecular formula. $(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$

Relative molecular mass. 1457

Graphic formula.



Chemical name. *O*-2-Deoxy-2-(methylamino)-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-*N*,*N*′-bis(aminoiminomethyl)-D-streptamine sulfate (2:3) (salt);CAS Reg. No. 3810-74-0.

Description. A white or almost white powder; odourless or with a slight odour.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS, and ether R.

Category. Antibiotic.

Storage. Streptomycin sulfate should be kept in a well-closed container and protected from moisture.

Labelling. The designation sterile Streptomycin sulfate indicates that the substance complies with the additional requirements for sterile Streptomycin sulfate and may be used for parenteral administration or for other sterile applications.

Additional information. Streptomycin sulfate is hygroscopic, but it is stable in air and on exposure to light.

Requirements

Definition. Streptomycin sulfate contains not less than 90.0% of $(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$ and not less than 720 International Units per mg, both calculated with reference to the dried substance.

Manufacture. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine-like substances. Carry out the test as described under 3.6 Test for histamine-like substances (vasodepressor substances) using, per kg of body weight, a solution containing 3 mg of streptomycin base in 1 ml of saline TS.

Identity tests

A. Dissolve 20 mg in 5 ml of water and boil for a few minutes with 10 drops of sodium hydroxide (1 mol/l) VS; add 3 drops of hydrochloric acid (~250 g/l) TS and 1 ml of ferric chloride (25 g/l) TS; an intense violet colour is produced.

B. Dissolve 0.1 g in 2 ml of water, add 1 ml of 1-naphthol TS1 and 2 ml of a mixture of equal volumes of sodium hypochlorite (~40 g/l) TS and water; a red colour is produced.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw4 when compared as described in 1.11 Colour of liquids.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 70 mg/g.

pH value. pH of a 0.25 g/ml solution in carbon-dioxide-free water R, 4.5–7.0.

Methanol. Transfer 0.2 g, accurately weighed, to a flask, dissolve in 5 ml of water and add 0.05 ml of sulfuric acid (0.05 mol/l) VS; connect the flask to a distillation apparatus, distil and collect about 2.5 ml of distillate in a 10-ml test-tube. Transfer the distillate to a conical flask, rinsing the test-tube twice with water, using 1 ml each time, and add 25 ml of potassium dichromate (0.0167 mol/l) VS. Cautiously add 10 ml of sulfuric acid (~1760 g/l) TS and heat the resulting solution for 30 minutes on a water-bath; cool and dilute to about 500 ml with water. Add 12.5 ml of potassium iodide (80 g/l) TS, allow to stand for 5 minutes, and then titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, added towards the end of the titration, the endpoint being reached when the dark blue colour turns pale green. Repeat the opera-

tion without the substance being tested; the difference between the volumes used for the two titrations represents the amount of sodium thiosulfate (0.1 mol/l) VS, equivalent to the methanol present. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 0.534 mg of CH_4O; the methanol content is not more than 40 mg/g, calculated as CH_4O.

Assay

For streptomycin sulfate. Dissolve about 0.10 g, accurately weighed, in sufficient water to produce 100 ml. To 5 ml add 5 ml of sodium hydroxide (0.2 mol/l) VS and heat in a water-bath for exactly 10 minutes. Cool in ice for exactly 5 minutes, add 3 ml of ferric ammonium sulfate TS2 and sufficient water to produce 25 ml, and mix. Exactly 20 minutes after the addition of the ferric ammonium sulfate TS2 measure the absorbance of a 1-cm layer at the maximum at about 525 nm, against a solvent cell containing a solution prepared in the same manner but omitting the substance being examined. Calculate the content of $(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$ in the substance, using the absorptivity value of 1.18 ($E^{1\%}_{1\,cm} = 11.8$).

For potency. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus subtilis* (NCTC 8236, or ATCC 11774) as the test organism, culture medium Cm1 with a final pH of 7.9–8.0, sterile phosphate buffer pH 8.0, TS1 or TS2, an appropriate concentration of streptomycin (usually between 5 and 20 IU), and an incubation temperature of 36–39 °C, or (*b*) *Bacillus subtilis* (ATCC 6633) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer pH 8.0, TS1 or TS2, an appropriate concentration of streptomycin (usually between 3 and 15 IU), and an incubation temperature of 35–37 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 720 IU per mg, calculated with reference to the dried substance.

Additional Requirements for Streptomycin Sulfate for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.25 IU of endotoxin RS per mg of streptomycin.

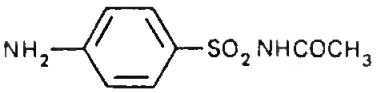
Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

SULFACETAMIDUM
SULFACETAMIDE

Molecular formula. $C_8H_{10}N_2O_3S$

Relative molecular mass. 214.2

Graphic formula.



Chemical name. *N*-Sulfanilylacetamide; *N*-[(4-aminophenyl)sulfonyl] acetamide; CAS Reg. No. 144-80-9.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; freely soluble in acetone R and methanol R; soluble in ethanol (~750 g/l) TS.

Category. Antiinfective agent.

Storage. Sulfacetamide should be kept in a well-closed container, protected from light.

Requirements

Definition. Sulfacetamide contains not less than 99.0% and not more than 101.0% of $C_8H_{10}N_2O_3S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from Sulfacetamide RS or with the *reference spectrum* of Sulfacetamide.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 0.10 g in 5 ml of ethanol (~750 g/l) TS, add about 0.2 ml of sulfuric acid (~1760 g/l) TS and heat; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

Melting range. 181–184 °C.

Heavy metals. For the preparation of the test solution use 1.0 g dissolved in a mixture of 10 ml of water and 1.0 ml of acetic acid (~300 g/l) TS, heat until dissolved, cool, and filter. Dilute to 40 ml with water and determine the heavy metals content as described under 2.2.3 Limit test for heavy metals, according to Method A; not more than 20 µg/g.

Solution in alkali. Dissolve 0.5 g in 10 ml of sodium hydroxide (1 mol/l) VS; the solution is clear or any opalescence produced is not more pronounced than that of opalescence standard TS2, and the solution is not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Solution in acid. A solution of 1.0 g in 10 ml of hydrochloric acid (1 mol/l) VS is clear or any opalescence produced is not more pronounced than that of opalescence standard TS2.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 20 volumes of chloroform R, 2 volumes of methanol R, and 1 volume of dimethylformamide R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in a mixture of 9 volumes of ethanol (~750 g/l) TS and 1 volume of ammonia (~260 g/l) TS containing (A) 2.5 mg of the test substance per ml, (B) 2.5 mg of sulfacetamide RS per ml, and (C) 12.5 µg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Spray the dried plate with sulfuric acid/ethanol TS, heat it at 105 °C for 30 minutes, and immediately expose it to nitrous fumes in a closed chamber for 15 minutes (the nitrous fumes may be generated by adding sulfuric acid (~700 g/l) TS drop by drop to a solution containing 10 g of sodium nitrite R and 3 g of potassium iodide R in 100 ml). Place the plate in a current of warm air for 15 minutes and spray it with N-(1-naphthyl)ethylenediamine hydrochloride/ethanol TS. If necessary, allow it to dry before repeating the spraying and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

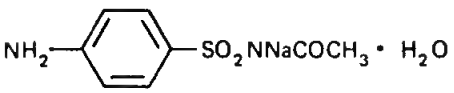
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 20 ml of hydrochloric acid (~250 g/l) TS and 50 ml of water, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 21.42 mg of $C_8H_{10}N_2O_3S$.

SULFACETAMIDUM NATRICUM
SULFACETAMIDE SODIUM

Molecular formula. $C_8H_9N_2NaO_3S,H_2O$

Relative molecular mass. 254.2

Graphic formula.



Chemical name. *N*-Sulfanilylacetamide monosodium salt monohydrate; *N*-[(4-aminophenyl)sulfonyl]acetamide monosodium salt monohydrate; CAS Reg. No. 6209-17-2 (monohydrate).

Other name. Sulfacylum-natrium.

Description. A white or yellowish white, crystalline powder; odourless.

Solubility. Soluble in 1.5 parts of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiinfective agent.

Storage. Sulfacetamide sodium should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Sulfacetamide sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Sulfacetamide sodium contains not less than 99.0% and not more than 101.0% of $C_8H_9N_2NaO_3S$, calculated with reference to the anhydrous substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Dissolve 1 g in 10 ml of water and add 2 ml of acetic acid (~300 g/l) TS; a white precipitate is produced. Collect the precipitate on a filter, wash it with cold water, and dry it at 105 °C. Carry out the examination with the dried residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from Sulfacetamide RS or with the *reference spectrum* of sulfacetamide. Keep the remaining precipitate for tests B and C.

B. Melting temperature of the precipitate obtained in test A, about 183 °C (sulfacetamide).

C. Dissolve 0.1 g of the precipitate obtained in test A in 5 ml of ethanol (~750 g/l) TS, add about 0.2 ml of sulfuric acid (~1760 g/l) TS, and heat; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, ignite 0.5 g and dissolve the residue in acetic acid (~60 g/l) TS.

Clarity and colour of solution. A solution of 0.5 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

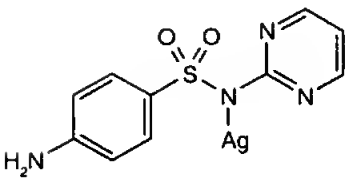
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not less than 60 mg/g and not more than 80 mg/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 8.0–9.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 50 volumes of 1-butanol R, 25 volumes of dehydrated ethanol R, 25 volumes of water, and 10 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions containing (A) 0.10 g of the test substance per ml, (B) 0.50 mg of sulfanilamide RS per ml, and (C) 0.25 mg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Spray the plate with 4-dimethylaminobenzaldehyde TS5. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Not more than one of any such spots is more intense than the spot obtained with solution C.

Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 50 ml of water and 20 ml of hydrochloric acid (~70 g/l) TS, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 23.62 mg of $C_8H_9N_2NaO_3S$.

SULFADIAZINUM ARGENTUM
SULFADIAZINE SILVER



$C_{10}H_9AgN_4O_2S$

Relative molecular mass. 357.1

Chemical name. N^1-2-Pyrimidinylsulfanilamide monosilver(1+) salt; 4-amino-*N*-2-pyrimidinylbenzenesulfonamide monosilver(1+) salt; CAS Reg. No. 22199-08-2.

Description. A white or almost white, crystalline powder.

Solubility. Sulfadiazine silver is practically insoluble in water and ethanol (~750 g/l) TS; slightly soluble in acetone R and ether R; soluble in ammonia (~260 g/l) TS.

Category. Anti-infective drug.

Storage. Sulfadiazine silver should be kept in a well-closed container, protected from light.

Requirements

Sulfadiazine silver contains not less than **98.0%** and not more than **102.0%** of $C_{10}H_9AgN_4O_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

Prepare the following residue to be used in tests A, B, and C: dissolve 0.5 g in 5 ml of nitric acid (~1000 g/l) TS, add 20 ml of water and 20 ml of sodium chloride (400 g/l) TS, mix, and filter. Neutralize the filtrate with sodium hydroxide (~80 g/l) TS using phenolphthalein/ethanol TS as indicator, and add 2.0 ml of acetic acid (~60 g/l) TS; a white precipitate is produced. Filter, wash the precipitate on the filter with water, and dry it at 105 °C for 1 hour.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfadiazine RS or with the *reference spectrum* of sulfadiazine.

B. About 10 mg of the residue yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing an orange-red precipitate.

C. Dissolve about 0.1 g of the residue in 3 ml of water, add 3 ml of sodium hydroxide (50 g/l) TS, shake, and filter. To a portion of the filtrate add 1 drop of copper(II) sulfate (160 g/l) TS; a yellowish green precipitate is produced that on standing turns to brownish red.

D. To about 0.1 g add 20 ml of water, 2 ml of nitric acid (~130 g/l) TS, and mix; a curdy, white precipitate is produced which is soluble in ammonia (~100 g/l) TS.

Loss on drying. Dry to constant mass at 80 °C; it loses not more than 5.0 mg/g.

pH value. Heat 1.0 g with 50 ml of carbon-dioxide-free water R to 70 °C for 5 minutes, cool rapidly, and filter. pH of the filtrate, 5.5–7.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 7 volumes of dichloromethane R, 4 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. (*Note*: Mix the dichloromethane and methanol before adding the ammonia.) Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A) dissolve 50 mg of Sulfadiazine silver in 3.0 ml of ammonia (~260 g/l) TS and dilute with sufficient methanol R to produce 10 ml. For solution (B) dilute 1.0 ml of solution A with a mixture containing 4 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS to produce 100 ml. Allow the spots to dry before development. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not larger and more intense than that obtained with solution B (1.0%).

Assay. Transfer into a stoppered flask about 0.5 g, accurately weighed, and dissolve in 8 ml of nitric acid (~130 g/l) TS. Add 50 ml of water and titrate with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the procedure without the Sulfadiazine silver being examined and make any necessary corrections.

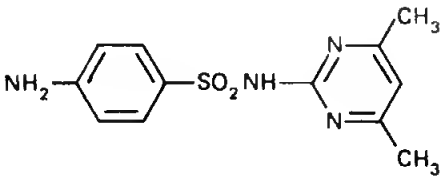
Each ml of ammonium thiocyanate (0.1 mol/l) VS is equivalent to 35.71 mg of $C_{10}H_9AgN_4O_2S$.

SULFADIMIDINUM
SULFADIMIDINE

Molecular formula. $C_{12}H_{14}N_4O_2S$

Relative molecular mass. 278.3

Graphic formula.



Chemical name. N^1-(4,6-Dimethyl-2-pyrimidinyl)sulfanilamide; 4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide; CAS Reg. No. 57-68-1.

Other names. Sulfadimezinum, sulfamethazine.

Description. A white or creamy white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS; soluble in acetone R; practically insoluble in ether R.

Category. Antibacterial drug.

Storage. Sulfadimidine should be kept in a well-closed container, protected from light.

Requirements

Definition. Sulfadimidine contains not less than 99.0% and not more than 100.5% of $C_{12}H_{14}N_4O_2S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfadimidine RS or with the *reference spectrum* of sulfadimidine.

B. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing an orange precipitate.

C. Boil gently 0.5 g with 1 ml of sulfuric acid (~700 g/l) TS until a precipitate is formed (about 2 minutes). Cool, add 15 ml of sodium hydroxide (~80 g/l) TS and extract the mixture with 20 ml of ether R. Filter the ether extract through a layer of anhydrous sodium sulfate R and evaporate to dryness on a water-bath. Melting temperature, about 153 °C (2-amino-4,6-dimethylpyrimidine).

D. Melting temperature, about 197 °C with decomposition.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Heat 2.0 g with 100 ml of carbon-dioxide-free water R at about 70 °C for 5 minutes, cool and filter. Titrate 25 ml of the filtrate with sodium hydroxide (0.1 mol/l) VS, using bromothymol blue/ethanol TS as indicator; not more than 0.2 ml is required to obtain the midpoint of the indicator (green).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 15 volumes of 1-butanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in acetone R containing (A) 10 mg of the test substance per ml and (B) 0.050 mg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, heat it at 105 °C for 10 minutes and spray it with 4-dimethylaminobenzaldehyde TS3. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in 50 ml of hydrochloric acid (~70 g/l) TS,

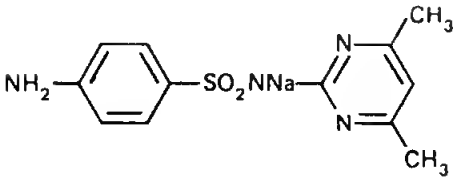
and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 27.83 mg of $C_{12}H_{14}N_4O_2S$.

SULFADIMIDINUM NATRICUM
SULFADIMIDINE SODIUM

Molecular formula. $C_{12}H_{13}N_4NaO_2S$

Relative molecular mass. 300.3

Graphic formula.



Chemical name. N^1-(4,6-Dimethyl-2-pyrimidinyl)sulfanilamide monosodium salt; 4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide monosodium salt; CAS Reg. No. 1981-58-4.

Description. A white or creamy white, crystalline powder; odourless or almost odourless.

Solubility. Soluble in 2.5 parts of water and in 60 parts of ethanol (~750 g/l) TS.

Category. Antibacterial drug.

Storage. Sulfadimidine sodium should be kept in a well-closed container, protected from light.

Additional information. Even in the absence of light, Sulfadimidine sodium is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Sulfadimidine sodium contains not less than 98.0% and not more than 101.0% of $C_{12}H_{13}N_4NaO_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Dissolve 0.1 g in 10 ml of water, acidify with hydrochloric acid (~70 g/l) TS, filter, and dry the residue at 105 °C. Carry out the examination with the dried residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfadimidine RS or with the *reference spectrum* of sulfadimidine.

B. Dissolve 0.1 g in 10 ml of water, add 2 ml of acetic acid (~300 g/l) TS, separate the precipitate by filtration, and wash with cold water. It yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a bright orange-red precipitate.

C. Boil gently 0.5 g with 1.5 ml of sulfuric acid (~700 g/l) TS until a precipitate is formed (about 2 minutes). Cool, add 15 ml of sodium hydroxide (~80 g/l) TS and extract the mixture with 20 ml of ether R. Filter the ether extract through a layer of anhydrous sodium sulfate R and evaporate to dryness on a water-bath. Melting temperature, about 153 °C (2-amino-4,6-dimethylpyrimidine).

D. When tested for sodium as described under 2.1 General identification tests yields the characteristic reactions. If reaction B is to be used, ignite a small quantity and dissolve the residue in acetic acid (~60 g/l) TS.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 3.3 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Yw4 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

pH value. pH of a 0.10 g/ml solution in carbon-dioxide-free water R, 10.0–11.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 15 volumes of 1-butanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 9 volumes of ethanol (~750 g/l) TS and 1 volume of ammonia (~260 g/l) TS containing (A) 10 mg of the test substance per ml and (B) 0.050 mg of sulfanilamide

RS per ml (for the preparation of solution A, first dissolve the test substance in a little ammonia (~260 g/l) TS, add 9 volumes of ethanol (~750 g/l), and then dilute to the required volume with the ethanol/ammonia mixture). After removing the plate from the chromatographic chamber, heat it at 105 °C for 10 minutes and spray it with 4-dimethylaminobenzaldehyde TS3. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

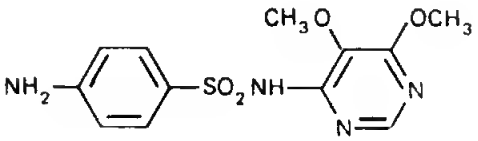
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 75 ml of water and 10 ml of hydrochloric acid (~420 g/l) TS, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 30.03 mg of $C_{12}H_{13}N_4NaO_2S$.

SULFADOXINUM
SULFADOXINE

Molecular formula. $C_{12}H_{14}N_4O_4S$

Relative molecular mass. 310.3

Graphic formula.



Chemical name. *N*[1]-(5,6-Dimethoxy-4-pyrimidinyl)sulfanilamide; 4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide; CAS Reg. No. 2447-57-6.

Description. A white or creamy white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS and in methanol R; practically insoluble in ether R.

Category. Antimalarial drug.

Storage. Sulfadoxine should be kept in a well-closed container, protected from light.

Requirements

Definition. Sulfadoxine contains not less than 99.0% and not more than 101.0% of $C_{12}H_{14}N_4O_4S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfadoxine RS or with the *reference spectrum* of sulfadoxine.

B. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing an orange-red precipitate.

C. Dissolve 50 mg in 3 ml of sodium hydroxide (0.1 mol/l) VS, heating slightly. Cool and add 1.0 ml of copper(II) sulfate (80 g/l) TS; a greenish yellow precipitate is produced, the colour of which changes to blue (distinction from certain other sulfonamides).

D. Melting temperature, about 199 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. For the preparation of the test solution, boil 2.5 g with 30 ml of water, cool and filter. Add 10 ml of nitric acid (~130 g/l) TS to the filtrate and proceed as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.1 mg/g.

Sulfates. For the preparation of the test solution, boil 2.5 g with 40 ml of water, cool and filter. Proceed with the nitrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.2 mg/g.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of hydrochloric acid (~70 g/l) TS is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Heat 1.0 g with 50 ml of carbon-dioxide-free water R at about 70 °C for 5 minutes, cool quickly to 20 °C, and filter; titrate 25 ml of the filtrate to pH 7.0 with sodium hydroxide (0.1 mol/l) VS; not more than 0.25 ml is required.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 15 volumes of 1-butanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 9 volumes of ethanol (~750 g/l) TS and 1 volume of ammonia (~260 g/l) TS containing (A) 10 mg of the test substance per ml and (B) 0.050 mg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, heat it at 105 °C for 10 minutes and spray it with 4-dimethylaminobenzaldehyde TS3. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

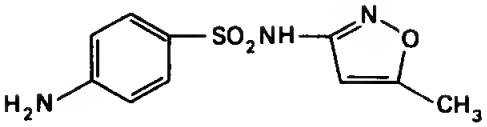
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 75 ml of water and 10 ml of hydrochloric acid (~250 g/l) TS, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 31.03 mg of $C_{12}H_{14}N_4O_4S$.

SULFAMETHOXAZOLUM
SULFAMETHOXAZOLE

Molecular formula. $C_{10}H_{11}N_3O_3S$

Relative molecular mass. 253.3

Graphic formula.



Chemical name. *N'*-(5-Methyl-3-isoxazolyl)sulfanilamide: 4-amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; CAS Reg. No. 723-46-6.

Description. A white or yellowish white, crystalline powder; odourless.

Solubility. Very slightly soluble in water; soluble in 50 parts of ethanol (~750 g/l) TS and in 3 parts of acetone R.

Category. Antibacterial.

Storage. Sulfamethoxazole should be kept in a well-closed container, protected from light.

Requirements

Definition. Sulfamethoxazole contains not less than 99.0% and not more than 101.0% of $C_{10}H_{11}N_3O_3S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfamethoxazole RS or with the *reference spectrum* of sulfamethoxazole.

B. Dissolve 5 mg in 0.5 ml of sodium hydroxide (~80 g/l) TS and add 5 ml of water. Add 0.1 g of phenol R, heat to boiling, cool, and add 1 ml of sodium hypochlorite (~40 g/l) TS; a golden yellow colour is immediately produced and is persistent.

C. About 0.1 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a red-orange precipitate.

Melting range. 168–172 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Solution in alkali. Dissolve 0.40 g in a mixture of 8.0 ml of water and 2.0 ml of sodium hydroxide (1 mol/l) VS; the solution is clear.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Heat 1.0 g with 50 ml of carbon-dioxide-free water R at about 70 °C for 5 minutes, cool quickly to 20 °C, and filter; titrate 25 ml of the filtrate, phenolphthalein/ethanol TS being used as indicator; not more than 0.35 ml of sodium hydroxide (0.1 mol/l) VS is required to obtain the midpoint of the indicator.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 20 volumes of chloroform R, 2 volumes of methanol R, and 1 volume of dimethylformamide R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 9 volumes of ethanol (~750 g/l) TS and 1 volume of ammonia (~260 g/l) TS containing (A) 2.5 mg of the test substance per ml and (B) 12.5 µg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Spray the dried plate with sulfuric acid/ethanol TS, heat at 105 °C for 30 minutes, and immediately expose to nitrous fumes in a closed chamber for 15 minutes (the nitrous fumes may be generated by adding sulfuric acid (~700 g/l) TS drop by drop to a solution containing 10 g of sodium nitrite R and 3 g of potassium iodide R in 100 ml). Place the plate in a current of warm air for 15 minutes and spray with *N*-(1-naphthyl)ethylenediamine hydrochloride/ethanol TS. If necessary, allow to dry before repeating the spraying and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 40 ml of water and 20 ml of glacial acetic acid R; add 15 ml of hydrochloric acid (~70 g/l) TS and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$.

Additional requirement for Sulfamethoxazole for parenteral use

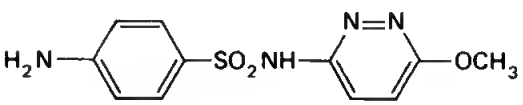
Complies with the monograph for "Parenteral preparations".

SULFAMETHOXYPYRIDAZINUM
SULFAMETHOXYPYRIDAZINE

Molecular formula. $C_{11}H_{12}N_4O_3S$

Relative molecular mass. 280.3

Graphic formula.



Chemical name. *N'*-(6-Methoxy-3-pyridazinyl)sulfanilamide; 4-amino-*N*-(6-methoxy-3-pyridazinyl)benzenesulfonamide; CAS Reg. No. 80-35-3.

Description. A white or yellowish white, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; soluble in acetone R.

Category. Antibacterial.

Storage. Sulfamethoxypyridazine should be kept in a well-closed container, protected from light.

Additional information. Sulfamethoxypyridazine becomes gradually coloured on exposure to light.

Requirements

Definition. Sulfamethoxypyridazine contains not less than 99.0% and not more than 101.0% of $C_{11}H_{12}N_4O_3S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfamethoxypyridazine RS or with the *reference spectrum* of sulfamethoxypyridazine.

B. About 0.05 g yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a bright orange-red precipitate.

C. To 20 mg add 10 ml of sulfuric acid (~100 g/l) TS, mix to dissolve and carefully add 0.1 ml of potassium bromate (50 g/l) TS; a yellow colour that changes to amber is produced and a brown precipitate is gradually formed.

Melting range. 180–183 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals. Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Acidity. Heat 1.0 g with 50 ml of carbon-dioxide-free water R at about 70 °C for 5 minutes, cool quickly to 20 °C, and filter; 25 ml of the filtrate requires for titration to pH 7.0 not more than 0.35 ml of sodium hydroxide (0.1 mol/l) VS.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 20 volumes of chloroform R, 2 volumes of methanol R, and 1 volume of dimethylformamide R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 9 volumes of ethanol (~750 g/l) TS and 1 volume of ammonia (~260 g/l) TS containing (A) 2.5 mg of the test substance per ml and (B) 12.5 µg of sulfanilamide RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated. Spray the dried plate with sulfuric acid/ethanol TS, heat at 105 °C for 30 minutes, and immediately expose to nitrous fumes in a closed chamber for 15 minutes (the nitrous fumes may be generated by adding sulfuric acid (~700 g/l) TS drop by drop to a solution containing 10 g of sodium nitrite R and 3 g of potassium iodide R in 100 ml). Place the plate in a current of warm air for 15 minutes and spray with *N*-(1-naphthyl)ethylenediamine hydrochloride/ethanol TS. If necessary, allow to dry before repeating the spraying and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

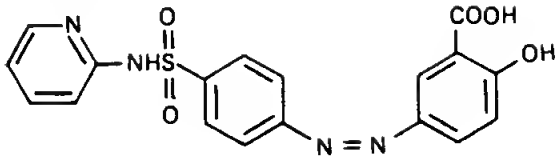
Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in 50 ml of hydrochloric acid (~70 g/l) TS, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 28.03 mg of $C_{11}H_{12}N_4O_3S$.

SULFASALAZINUM
SULFASALAZINE

Molecular formula. $C_{18}H_{14}N_4O_5S$

Relative molecular mass. 398.4

Graphic formula.



Chemical name. 5-[[*p*-(2-Pyridylsulfamoyl)phenyl]azo]salicylic acid; 2-hydroxy-5-[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo]benzoic acid; CAS Reg. No. 599-79-1.

Other name. Salazosulfapyridine.

Description. A bright yellow to brownish yellow powder; odourless.

Solubility. Practically insoluble in water, and ether R; very slightly soluble in ethanol (~750 g/l) TS; soluble in alkali hydroxides.

Category. Antibacterial drug.

Storage. Sulfasalazine should be kept in a tightly closed container, protected from light.

Additional information. Sulfasalazine melts at about 255 °C with decomposition.

Requirements

Definition. Sulfasalazine contains not less than 93.0% and not more than 103.0% of $C_{18}H_{14}N_4O_5S$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from sulfasalazine RS or with the *reference spectrum* of sulfasalazine.

B. The absorption spectrum of the solution as prepared in the assay below, when observed between 230 nm and 600 nm, exhibits maxima and minima at the same wavelengths as does the absorption spectrum of a solution of sulfasalazine RS prepared in a similar manner.

C. Dissolve 0.10 g in a mixture of 1.0 ml of ethanol (~750 g/l) TS and 4 ml of hydrochloric acid (~70 g/l) TS, then add 0.20 g of zinc R powder. Heat on a water-bath for 5 minutes and filter. To 1.0 ml of the nitrate add 0.1 ml of ferric chloride (25 g/l) TS; a red colour is produced. To 1.0 ml of the filtrate add 1.0 ml of sodium nitrite (10 g/l) TS and allow to stand for 1 minute. Then add 2.0 ml of sodium hydroxide (~80 g/l) TS and 0.1 ml of 2-naphthol TS1; a strong red colour is produced.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Digest 2.0 g with 100 ml of water at 70 °C for 5 minutes. Cool immediately to room temperature and filter. To 25 ml of the filtrate (keep the remaining filtrate for the limit test for sulfates) add 1 ml of nitric acid (~1000 g/l) TS and allow to stand for 5 minutes. Filter through a fine-texture filter-paper and proceed with the filtrate as described under 2.2.1 Limit test for chlorides; the chloride content is not more than 0.14 mg/g.

Sulfates. To 25 ml of the filtrate retained from the limit test for chlorides add 1.5 ml of hydrochloric acid (2 mol/l) VS and allow to stand for 5 minutes. Filter through a fine-texture filter-paper and proceed with the filtrate as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 0.4 mg/g.

Sulfated ash. Not more than 5.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Assay. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R2 as the coating substance and a mixture of 4 volumes of chloroform R, 1 volume of 1-butanol R, 1 volume of acetone R, and 1 volume of formic acid (~1080 g/l) TS as the mobile phase, allowing the chamber to equilibrate for 18 hours. Apply separately to the plate 10 µl of each of 2 solutions in dimethylformamide R containing (A) 12 mg of the test substance per ml, (B) 12 mg of sulfasalazine RS per ml, and (C) 10 µl of dimethylformamide R to serve as a blank. Dry the spotted plate thoroughly at room temperature to remove traces of dimethylformamide and develop the plate over a distance of 15 cm. After removing the plate from the chromatographic chamber, mark the solvent front and allow the solvent to evaporate. Locate the spots by examination of the chromatogram in ultraviolet light (254 nm) and mark the sulfasalazine spots at an R_f value of about 0.6, excluding an adjacent impurity band. Remove quantitatively and separately the silica gel mixture containing each of the 3 spots from solutions A, B and C, transferring them to separate glass-stoppered centrifuge tubes. (NOTE: The dimethylformamide R employed in the next step should be obtained from a recently opened bottle and should contain less than

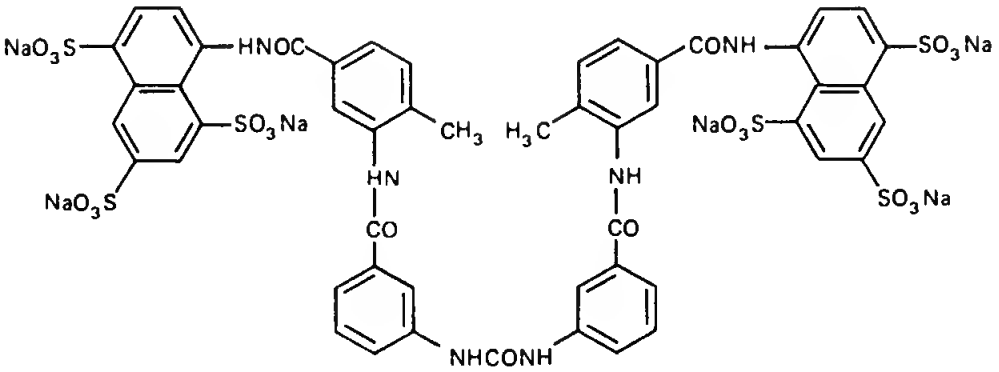
10 mg of water per g. Dimethylformamide dried over alkali pellets is not acceptable.) Add 10.0 ml of dimethylformamide R to each tube, insert the stopper, shake for 10 minutes, and centrifuge until clear. Measure the absorbance of the solutions in a 1-cm layer at the maximum at about 406 nm against a solvent cell containing the blank obtained from solution C. Calculate the amount of $C_{18}H_{14}N_4O_5S$ in the substance being tested (solution A) by comparison with sulfasalazine RS (solution B).

SURAMINUM NATRICUM
SURAMIN SODIUM

Molecular formula. $C_{51}H_{34}N_6Na_6O_{23}S_6$

Relative molecular mass. 1429

Graphic formula.



Chemical name. Hexasodium 8,8′-[ureylenebis[*m*-phenylenecarbonylimino(4-methyl-*m*-phenylene)carbonylimino]]di-1,3,5-naphthalenetrisulfonate; hexasodium 8,8′-[carbonylbis[imino-3,1-phenylenecarbonylimino(4-methyl-3,1-phenylene)carbonylimino]]bis[1,3,5-naphthalenetrisulfonate]; CAS Reg. No. 129-46-4.

Other name. Nagananinum.

Description. A white, pinkish white or cream-coloured, crystalline powder; odourless.

Solubility. Very soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antifilarial drug; antitrypanosomal drug.

Storage. Suramin sodium should be kept in a tightly closed container, protected from light, and stored in a cool place.

Additional information. Suramin sodium is very hygroscopic and discolours on exposure to light.

Requirements

Definition. Suramin sodium contains not less than 96.0% and not more than 100.5% of $C_{51}H_{34}N_6Na_6O_{23}S_6$, calculated with reference to the anhydrous substance.

Manufacture. The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Therapeutic potency. For therapeutic potency, the sample is tested in mice infected with a strain of *Trypanosoma equiperdum*, or other suitable species of trypanosome sensitive to suramin sodium. The test may be carried out as follows: Inoculate at least 10 mice with the trypanosomes. After forty-eight hours examine the blood of each mouse microscopically and estimate the number of trypanosomes per ml in the blood of each mouse. The number should lie between 1000 and 20000. The estimate may be made by examining a thin film of the blood in the form of a cover-slip preparation, and counting the trypanosomes in at least 10 microscopic fields with an area of 0.12 mm^2. The presence of 1 to 20 trypanosomes in each of two fields corresponds approximately to a content of 1000 to 20000 trypanosomes per ml.

Inject into 10 of the infected mice, intravenously, 0.16 ml of a 50 mg/l solution of the sample in freshly distilled water per g of body mass. Examine the blood of each mouse microscopically, using a microscope with a 4-mm objective, on the first and third days after the injection. If no trypanosomes are found in the blood of 5 or more mice when 20 fields are examined on the third day, the sample passes the test. If trypanosomes are found under these conditions in the blood of more than 5 mice, repeat the test. The sample passes the test if no trypanosomes are found under these conditions in the blood of not less than 50% of the total number of mice treated.

Identity tests

A. Dissolve 20 mg in 2.0 ml of water, add 1.0 ml of hydrochloric acid (~70 g/l) TS, and heat on a water-bath for 5 minutes. Cool, add 0.25 ml of sodium nitrite (10 g/l) TS, allow to stand for 1 minute, then add 1.0 ml of sodium hydroxide (~80 g/l) TS and 0.15 ml of 2-naphthol TS1; a red colour is produced.

B. In a porcelain crucible, mix 20 mg with 0.10 g of anhydrous sodium carbonate R and heat until the gas evolution has ceased. Cool, dissolve the residue in 4 ml of hydrochloric acid (~70 g/l) TS, and filter. To 2.0 ml of the nitrate add 0.25 ml of barium chloride (50 g/l) TS; a white precipitate is produced.

C. Dissolve 0.05 g in 2.0 ml of water and add 0.25 ml of glacial acetic acid R; it yields reaction B characteristic of sodium as described under 2.1 General identification tests.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Chlorides. Dissolve 0.5 g in 10 ml of water, add 5 ml of nitric acid (~130 g/l) TS, 5 ml of silver nitrate (0.1 mol/l) VS, and 3 ml of nitrobenzene R, and shake vigorously. Titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS, using 2 ml of ferric ammonium sulfate (45 g/l) TS as indicator; not less than 3.6 ml of ammonium thiocyanate (0.1 mol/l) VS are required.

Sulfates. Dissolve 0.50 g in 20 ml of water, add 3 ml of hydrochloric acid (~250 g/l) TS, and proceed as described under 2.2.2 Limit test for sulfates; the sulfate content is not more than 1 mg/g.

Clarity of solution. A solution of 0.50 g in 10 ml of carbon-dioxide-free water R is clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.2 g of the substance; the water content is not more than 0.10 g/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 5.5–7.0.

Free amines. Dissolve 5 g in 30 ml of water and add 30 ml of hydrochloric acid (1 mol/l) VS. Titrate at a temperature between 15 and 20 °C with sodium nitrite (0.1 mol/l) VS, stirring vigorously, until a blue colour is obtained on starch/iodide paper R. The end-point of the titration is reached when the blue colour is reproduced after the titrated solution has been allowed to stand for 1 minute. The titration can also be performed electrometrically. Repeat the operation without the substance to be examined. The difference in volume between the two titrations does not exceed 0.4 ml.

Assay. To about 0.5 g, accurately weighed, add 12 ml of sulfuric acid (~700 g/l) TS, and boil under a reflux condenser for 1 hour; cool, and dilute to about 100 ml with water. Add 1 g of potassium bromide R and titrate at a temperature between 15 and 20 °C with sodium nitrite (0.1 mol/l) VS, stirring vigor-

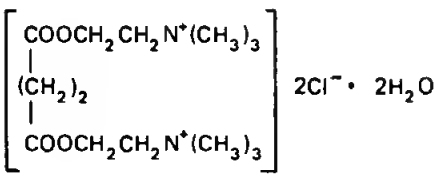
ously until a blue colour is obtained on starch/iodide paper R. The end-point of the titration is reached when the blue colour is reproduced after the titrated solution has been allowed to stand for 1 minute. The titration can also be performed electrometrically. Repeat the operation without the substance to be examined. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 23.82 mg of $C_{51}H_{34}N_6Na_6O_{23}S_6$.

SUXAMETHONII CHLORIDUM
SUXAMETHONIUM CHLORIDE

Molecular formula. $C_{14}H_{30}Cl_2N_2O_4,2H_2O$

Relative molecular mass. 397.3

Graphic formula.



Chemical name. Choline chloride, succinate (2:1), dihydrate; 2,2′-[(1,4-dioxo-1,4-butanediyl)bis(oxy)]bis[*N*,*N*,*N*-trimethylethanaminium] dichloride, dihydrate; 2,2′-succinyldioxybis(ethyltrimethylammonium) dichloride, dihydrate; CAS Reg. No. 6101-15-1 (dihydrate).

Other name. Succinylcholine chloride.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Soluble in 1 pan of water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Muscle relaxant.

Storage. Suxamethonium chloride should be kept in a tightly closed container, protected from light.

Additional information. Suxamethonium chloride is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Suxamethonium chloride contains not less than 98.0% and not more than 101.0% of $C_{14}H_{30}Cl_2N_2O_4$, calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 25 mg in 1 ml of water, add 0.1 ml of cobalt(II) chloride (5 g/l) TS and 0.1 ml of potassium ferrocyanide (45 g/l) TS; an emerald green colour is produced.

B. Dissolve 0.05 g in 10 ml of water, add 10 ml of sulfuric acid (~100 g/l) TS and 30 ml of ammonium reineckate (10 g/l) TS; a pink precipitate is produced. Allow to stand for 30 minutes, filter and wash with water, then with ethanol (~750 g/l) TS and with ether R. Dry the residue at 80 °C; melting temperature, about 183 °C.

C. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.15 g of the substance; not less than 80 mg/g and not more than 100 mg/g.

pH value. pH of a 10 mg/ml solution, 4.0–5.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R2 as the coating substance and a mixture of 4 volumes of 1-butanol R, 1 volume of acetic acid (~300 g/l) TS, and 5 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions containing (A) 5.0 mg of the test substance per ml and (B) 0.10 mg of the test substance per ml. Develop the plate for 2½ hours. After removing the plate from the chromatographic chamber, dry it at 90 °C for 15 minutes, allow it to cool, spray it with potassium iodoplatinate TS2 and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.3 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 30 ml of acetic anhydride R and 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-

aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$.

Additional requirements for Suxamethonium chloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 2.0 IU of endotoxin RS per mg.

TALCUM
TALC

Chemical name. Talc; talc $[Mg_3H_2(SiO_3)_4]$; CAS Reg. No. 14807-96-6.

Description. A white or almost white, very fine, homogeneous, unctuous powder; odourless.

Solubility. Practically insoluble in water, dilute solutions of acids and alkalis, ethanol (~750 g/l) TS, and ether R.

Category. Tablet and capsule lubricant; glidant; diluent.

Storage. Talc should be kept in a well-closed container.

Additional information. Talc adheres readily to the skin. Attention should be paid to its microbiological quality since it is of mineral origin.

Requirements

Definition. Talc is a powdered, natural hydrate of magnesium silicate that may contain variable proportions of aluminium and iron.

Identity tests

A. Transfer 0.5 g to a metal crucible, add 1 g of potassium nitrate R and 3 g of anhydrous sodium carbonate R, mix, and heat until melted. Allow to cool, add 20 ml of boiling water, mix, and filter. Wash the filter with 50 ml of water. Take up the residue with a mixture of 0.5 ml of hydrochloric acid (~420 g/l) TS and 5 ml of water and filter. To the filtrate add 1 ml of ammonia (~260 g/l) TS and 1 ml of ammonium chloride (100 g/l) TS and filter. To this filtrate add 1 ml of disodium hydrogen phosphate (100 g/l) TS; a white, crystalline precipitate is produced.

B. Using a copper wire mix 0.1 g with 10 mg of sodium fluoride R placed in a lead or platinum crucible, and add a few drops of sulfuric acid (~1760 g/l) TS to obtain a thin slurry. Cover the crucible with a thin, transparent, plastic plate from which a drop of water is suspended and warm gently; a white ring is produced around the drop of water within a short time.

Microscopic examination. Irregular plates, length up to 50 µm, are observed. Talc is free from microscopic asbestos fibres. A 1 mg/ml solution of methylthioninium chloride R in ethanol (~750 g/l) TS does not notably stain the particles.

Arsenic and heavy metals. Transfer 10 g to a 250-ml flask, add 50 ml of hydrochloric acid (0.5 mol/l) VS, attach a reflux condenser, and heat on a water-bath for 30 minutes. Cool, transfer the mixture to a beaker, and allow the undissolved material to settle. Decant the supernatant liquid through a thick, strong filter-paper of medium grade into a 100-ml volumetric flask, retaining as much as possible of the undissolved material in the beaker. Wash the beaker with three 10-ml portions of hot water, decanting each washing through the same filter. Finally, wash the filter with 15 ml of hot water, cool the filtrate and dilute to volume, and mix. Use this solution for the following tests:

— Use 10 ml and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 3 µg/g.

— Use 5 ml and proceed as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 40 µg/g.

Carbonates. Suspend 0.25 g in 40 ml of sulfuric acid (~100 g/l) TS; no effervescence is observed.

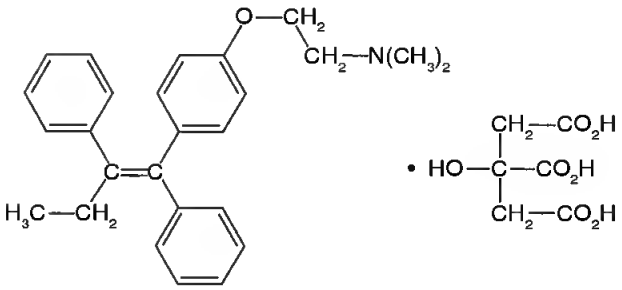
Acid-soluble substances. Digest 1.0 g with 20 ml of hydrochloric acid (~70 g/l) TS at 50 °C for 15 minutes, cool, add sufficient water to produce 50 ml, and filter. (If the filtrate is not clear, centrifuge it.) To 25 ml of the clear solution (keep the remaining solution for "Iron"), add 1 ml of sulfuric acid (~100 g/l) TS, and evaporate to dryness. Ignite the residue at 800 ± 25 °C and weigh; not more than 20 mg/g.

Iron. Acidify 10 ml of the filtrate obtained in the test above for "Acid-soluble substances" with hydrochloric acid (~70 g/l) TS and add 1 ml of potassium ferrocyanide (45 g/l) TS; no blue colour is observed.

Reaction and soluble substances. Boil 10 g with 50 ml of water for 30 minutes, adding water from time to time to maintain approximately the original volume, and filter; the filtrate is neutral to litmus paper R. Evaporate 20 ml of the filtrate to dryness and dry the residue at 105 °C for 1 hour; the mass of the residue does not exceed 4 mg (1 mg/g).

Loss on ignition. Ignite 1.0 g at 1000 °C to constant mass; it loses not more than 65 mg/g.

TAMOXIFENI CITRAS
TAMOXIFEN CITRATE



$C_{26}H_{29}NO,C_6H_8O_7$

Relative molecular mass. 563.6

Chemical name. (*Z*)-2-[*p*-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine citrate (1:1); (*Z*)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1); CAS Reg. No. 54965-24-1.

Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water and acetone R; soluble in methanol R.

Category. Antiestrogen.

Storage. Tamoxifen citrate should be kept in a well-closed container, protected from light.

Requirements

Tamoxifen citrate contains not less than **99.0%** and not more than the equivalent of **101.0%** of $C_{26}H_{29}NO,C_6H_8O_7$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from tamoxifen citrate RS or with the *reference spectrum* of tamoxifen citrate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of toluene R and 1 volume of triethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of two solutions in methanol R containing (A) 10 mg of Tamoxifen citrate per ml and (B) 10 mg of tamoxifen citrate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B

C. To 10 mg add 4 ml of pyridine R and 2 ml of acetic anhydride R, and shake; a yellow colour is immediately produced. Heat on a water-bath for 2 minutes; a light pink to red colour is produced.

D. Melting temperature, about 142 °C with decomposition.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

***E*-isomer and related substances.** Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (20 cm × 5 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 400 volumes of acetonitrile R and 600 volumes of water containing 0.9 g/l of sodium dihydrogen phosphate R and 4.8 g/l of *N*,*N*-dimethyloctylamine R adjusted to pH 3.0 with phosphoric acid (~105 g/l) TS.

Prepare the following solutions in the mobile phase: for solution (A) use 1.0 mg of Tamoxifen citrate per ml; for solution (B) use 1.0 mg of tamoxifen citrate *E*-isomer RS per ml; for solution (C) dilute 1 volume of solution A to 100 volumes with the mobile phase; and for solution (D) dilute 1 volume of solution B to 100 volumes with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 240 nm, the instrument being fitted with a low volume flow cell (10 µl).

Equilibrate the column with the mobile phase at a flow rate of 1.0 ml per minute for about 30 minutes.

Make three replicate injections of 10 µl each of solution D, adjusting the sensitivity of the system so that the height of the peak is not less than 40% of the full scale of the recorder.

Inject alternately 10 µl each of solutions A, C, and D. The test is not valid unless the peak obtained with solution D elutes prior to that for solution C, and with solution A there is baseline separation between all the peaks.

Measure the areas of the peak responses obtained in the chromatograms from solutions A, C, and D, and calculate the content of the related substances as a percentage. Calculate the content of *E*-isomer comparing the peaks obtained with solutions A and D; not more than 10 mg/g. In the chromatogram obtained with solution A, the area of any peak, other than the peak due to the *E*-isomer obtained with solution D, is not greater than half that of the peak obtained with solution C (0.5%). Furthermore, the sum of the areas is not greater than the peak obtained with solution C (1%).

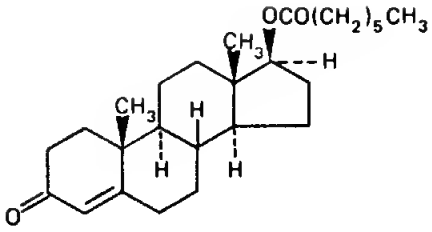
Assay. Dissolve about 1 g, accurately weighed, in 150 ml of glacial acetic acid R1, add 0.25 ml of 1-naphtholbenzein/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 56.36 mg of $C_{26}H_{29}NO,C_6H_8O_7$.

TESTOSTERONI ENANTAS
TESTOSTERONE ENANTATE

Molecular formula. $C_{26}H_{40}O_3$

Relative molecular mass. 400.6

Graphic formula.



Chemical name. Testosterone, heptanoate; 17β-[(1-oxoheptyl)oxy]androst-4-en-3-one; CAS Reg. No. 315-37-7.

Description. A white or creamy white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; very soluble in ethanol (~750 g/l) TS, ether R, and acetone R.

Category. Androgen.

Storage. Testosterone enantate should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 15 °C.

Additional information. Testosterone enantate melts at about 37 °C.

Requirements

Definition. Testosterone enantate contains not less than 97.0% and not more than 103.0% of $C_{26}H_{40}O_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from testosterone enantate RS or with the *reference spectrum* of testosterone enantate.

B. See the test described below under "Related substances". The principal spot obtained with solution C corresponds in position, appearance, and intensity with that obtained with solution D.

C. Suspend 5 mg in 2.0 ml of a mixture prepared by previously cooling 2 volumes of sulfuric acid (~1760 g/l) TS and 1 volume of ethanol (~750 g/l) TS, then place it in a water-bath; a greenish yellow fluorescence develops

that changes to orange, whereas the walls of the tube take on a dichroic blue colour, changing to red below a certain depth.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +77$ to $+83\,°$.

Loss on drying. Dry to constant weight at ambient temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 5.0 mg/g.

Free heptanoic acid. Dissolve 0.5 g, accurately weighed, in 10 ml of ethanol (~750 g/l) TS, previously neutralized to bromothymol blue/ethanol TS, and titrate immediately with sodium hydroxide (0.01 mol/l) VS, using bromothymol blue/ethanol TS as indicator; not more than 0.6 ml of sodium hydroxide (0.01 mol/l) VS is required to obtain the midpoint of the indicator (green).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 92 volumes of dichloroethane R, 8 volumes of methanol R, and 0.5 volume of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 20 mg of the test substance per ml; (B) 0.20 mg of the test substance per ml; (C) 1.0 mg of the test substance per ml and (D) 1.0 mg of testosterone enantate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and heat it at 110 °C for 10 minutes. Spray the hot plate with sulfuric acid/ethanol TS, again heat it at 110 °C for 10 minutes, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 20 mg, accurately weighed, in dehydrated ethanol R to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 241 nm. Calculate the amount of $C_{26}H_{40}O_3$ in the substance being tested by comparison with testosterone enantate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of a 10 µg/ml solution of testosterone enantate RS in dehydrated ethanol R should be 0.42 ± 0.02.

Additional requirements for Testosterone enantate for parenteral use

Complies with the monograph for "Parenteral preparations".

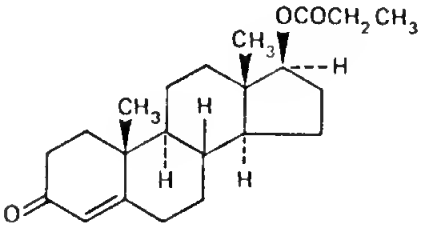
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 3.5 IU of endotoxin RS per mg.

TESTOSTERONI PROPIONAS
TESTOSTERONE PROPIONATE

Molecular formula. $C_{22}H_{32}O_3$

Relative molecular mass. 344.5

Graphic formula.



Chemical name. 17β-(1-Oxopropoxy)androst-4-en-3-one; 17β-hydroxyandrost-4-en-3-one propionate; CAS Reg. No. 57-85-2.

Description. Colourless or slightly yellowish crystals or a white or slightly yellowish powder; odourless.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS and ether R; soluble in vegetable oils.

Category. Androgen.

Storage. Testosterone propionate should be kept in a well-closed container, protected from light.

Requirements

Definition. Testosterone propionate contains not less than 97.0% and not more than 102.0% of $C_{22}H_{32}O_3$, calculated with reference to the dried substance.

Identity tests

- Either test A or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from testosterone propionate RS or with the *reference spectrum* of testosterone propionate.

B. See the test described below under "Related substances". The principal spot obtained with solution C corresponds in position, appearance, and intensity with that obtained with solution D.

C. Melting temperature, about 121 °C.

Specific optical rotation. Use a 10 mg/ml solution in dioxan R; $[\alpha]_D^{20\,°C} = +81$ to +91 °.

Solution in ethanol. A solution of 0.50 g in 10 ml of ethanol (~750 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 92 volumes of dichloroethane R, 8 volumes of methanol R, and 0.5 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 20 mg of the test substance per ml; (B) 0.20 mg of the test substance per ml; (C) 1.0 mg of the test substance per ml and (D) 1.0 mg of testosterone propionate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and heat at 110 °C for 10 minutes. Spray the hot plate with sulfuric acid/ethanol TS, again heat it at 110 °C for 10 minutes, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

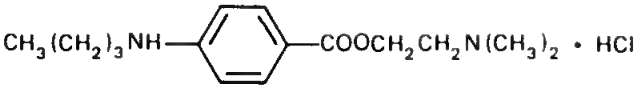
Assay. Dissolve about 20 mg, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml; dilute 5.0 ml of this solution to 100 ml with the same solvent. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 241 nm. Calculate the amount of $C_{22}H_{32}O_3$ in the substance being tested by comparison with testosterone propionate RS, similarly and concurrently examined. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.50 ± 0.03.

TETRACAINI HYDROCHLORIDUM
TETRACAINE HYDROCHLORIDE

Molecular formula. $C_{15}H_{24}N_2O_2,HCl$

Relative molecular mass. 300.8

Graphic formula.



Chemical name. 2-(Dimethylamino)ethyl *p*-(butylamino)benzoate monohydrochloride; 2-(dimethylamino)ethyl 4-(butylamino)benzoate monohydrochloride; CAS Reg. No. 136-47-0.

Other names. Amethocaine hydrochloride; dicainum.

Description. A white, crystalline powder; odourless.

Solubility. Soluble in about 8 parts of water; soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Local anaesthetic.

Storage. Tetracaine hydrochloride should be kept in a tightly closed container, protected from light.

Additional information. Tetracaine hydrochloride is hygroscopic; it has a slightly bitter taste and causes local numbness after being placed on the tongue. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.
Tetracaine hydrochloride melts at about 148 °C or may exist in either of the two polymorphic forms, one of which melts at 134 °C and the other at 139 °C. Mixtures of the forms melt within the range 134–147 °C.

Requirements

Definition. Tetracaine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{15}H_{24}N_2O_2$,HCl, calculated with reference to the dried substance.

Identity tests

A. Dissolve 0.2 g in 10 ml of water and add 1 ml of ammonium thiocyanate (75 g/l) TS. Collect the precipitate on a filter, recrystallize from water, and dry it at 80 °C for 2 hours; melting temperature, about 131 °C.

B. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 4.5–6.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 80 volumes of dibutyl ether R, 16 volumes of hexane R, and 4 volumes of glacial acetic acid R as the mobile phase. Place the plate in the chromatographic chamber, dipping it about 5 mm into the liquid. After the solvent has reached a height of about 12 cm, remove the plate from the chromatographic chamber and dry it for a few minutes in a current of warm air. Allow it to cool and apply separately to the plate 5 µl of each of 2 solutions containing (A) 0.10 g of the test substance per ml and (B) 0.050 mg of 4-aminobenzoic acid R per ml. Allow the solvent front to ascend 10 cm above the line of application. After removing the plate from the chromatographic chamber, dry it at 105 °C for 10 minutes and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. The principal spot remains on the baseline.

Assay. Carry out the assay as described under 2.7 Nitrite titration, using about 0.5 g, accurately weighed, dissolved in a mixture of 50 ml of water and 5 ml of hydrochloric acid (~420 g/l) TS, and titrate with sodium nitrite (0.1 mol/l) VS. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 30.08 mg of $C_{15}H_{24}N_2O_2$,HCl.

Additional requirements for Tetracaine hydrochloride for sterile use

Complies with 3.2.1 Test for sterility of non-injectable preparations.

TETRACYCLINI HYDROCHLORIDUM

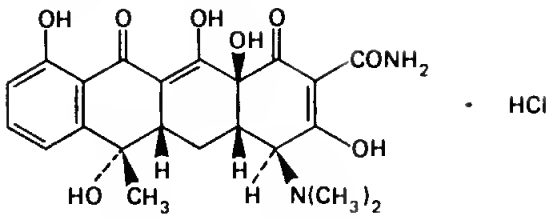
TETRACYCLINE HYDROCHLORIDE

Tetracycline hydrochloride (non-injectable)
Tetracycline hydrochloride, sterile

Molecular formula. $C_{22}H_{24}N_2O_8$,HCl

Relative molecular mass. 480.9

Graphic formula.



Chemical name. (4*S*,4a*S*,5a*S*,6*S*,12α*S*)-4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; [4*S*-(4α,4aα,5aα,6β, 12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride; CAS Reg. No. 64-75-5.

Description. A yellow, crystalline powder; odourless.

Solubility. Soluble in 10 parts of water and in 100 parts of ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Antibiotic.

Storage. Tetracycline hydrochloride should be kept in a tightly closed container, protected from light.

Labelling. The designation sterile Tetracycline hydrochloride indicates that the substance complies with the additional requirements for sterile Tetracycline hydrochloride and may be used for parenteral administration or for other sterile preparations.

Additional information. Tetracycline hydrochloride decomposes rapidly in solutions below pH 2, and less rapidly in solutions above pH 7. Even in the absence of light, Tetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Tetracycline hydrochloride contains when tested according to assay A not less than 96.0% and not more than 102.0% of $C_{22}H_{24}N_2O_8$,HCl, and when tested according to assay B not less than 950 International Units per mg, both calculated with reference to the dried substance.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, but using an unlined chamber and a cellulose coating prepared as follows: To

0.275 g of carbomer R add 120 ml of water, let the mixture stand for 1 hour while shaking it from time to time; then add gradually while stirring a sufficient volume of sodium hydroxide (~80 g/l) TS to adjust to pH 7.0. To this mixture add 30 g of cellulose R1 and a sufficient quantity of water (usually 60–80 ml) to obtain a coating substance of suitable consistency. Coat the plates with a layer 0.4 mm thick, and allow them to dry at room temperature. The plates thus coated are used after a suitable treatment both for the identity test and the test of "related substances". For the identity test spray the plate with phosphate/citrate buffer pH 4.5, TS, until traces of moisture appear. Dry the plate at 50 °C for 30 minutes.

Prepare the following solutions immediately before use while protected from bright light: Dissolve 5.0 mg of the test substance, 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 ml; this constitutes solution A. Dissolve 5.0 mg of chlortetracycline hydrochloride RS and 5.0 mg of oxytetracycline hydrochloride RS in sufficient methanol R to produce 10 ml; this constitutes solution B. Dissolve 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 ml; this constitutes solution C.

Apply separately to the plate 1 µl of each of solutions A, B and C, and spray it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity appear (about 8 ml).

Pour the mobile phase consisting of a mixture of 60 volumes of ethyl acetate R, 30 volumes of acetone R, and 6 volumes of water in the unlined chromatographic chamber. Place the plate in the chamber in such a manner that it is not in contact with the mobile phase. Allow the plate to become impregnated with the vapours for 1 hour. Then dip the plate into the mobile phase and allow the chromatogram to develop to a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram immediately in ultraviolet light (365 nm). Three principal clearly separated spots are obtained with solution A corresponding in position, appearance, and intensity with those obtained with solution C, two of which correspond with the spots obtained with solution B.

B. To about 1 mg add 2 ml of sulfuric acid (~1760 g/l) TS; a red-violet colour is produced which on the addition of 0.1 ml of water changes to yellow.

C. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 10 mg/ml solution in hydrochloric acid (0.01 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = –239 to –258 °.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 20 mg/g.

pH value. pH of a 10 mg/ml solution, 1.8–2.8.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate as prepared under the identity test A. To a sufficient volume of disodium edetate (0.1 mol/l) VS add sodium hydroxide (~80 g/l) TS to adjust to pH 7.0, and use this solution to spray the plate uniformly until traces of moisture appear. Dry the plate at 50 °C for 30 minutes.

Prepare the following solutions immediately before use, protecting them from bright light: Dissolve 0.10 g of the test substance in sufficient methanol R to produce 10 ml; this constitutes solution A. Dilute 2.5 ml of solution A to 10.0 ml with methanol R; this constitutes solution B. Dissolve 5.0 mg of 4-epianhydrotetracycline hydrochloride RS insufficient methanol R to produce 20 ml; this constitutes solution K. Dilute 2 ml of solution K to 10 ml with methanol R; this constitutes solution C. Dissolve 5.0 mg of 4-epitetracycline hydrochloride RS in sufficient methanol R to produce 8 ml; this constitutes solution L. Dilute 2 ml of solution L to 10 ml with methanol R; this constitutes solution D. Dissolve 5.0 mg of anhydrotetracycline hydrochloride RS in sufficient methanol R to produce 20 ml; this constitutes solution M. Dilute 2 ml of solution M to 10 ml with methanol R; this constitutes solution E. Dissolve 20 mg of chlortetracycline hydrochloride RS in sufficient methanol R to produce 20 ml; this constitutes solution N. Dilute 2 ml of solution N to 10 ml with methanol R; this constitutes solution F. Dissolve 10 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 20 ml; this constitutes solution P. Mix together 0.5 ml of each of the following solutions K, L, M, N and P; this constitutes solution G.

Apply separately to the plate 1 µl of each of solutions A, B, C, D, E, F, and G, and spray it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity appear (about 8 ml).

As the mobile phase, use a mixture of 60 volumes of ethyl acetate R, 30 volumes of acetone R, and 6 volumes of water. Allow the chromatogram to develop to a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram immediately in ultraviolet light (365 nm). The spot corresponding to 4-epitetracycline hydrochloride obtained with solution B is not more intense than that obtained with solution D (5% of 4-epitetracycline hydrochloride). The spots corresponding to 4-epianhydrotetracycline

hydrochloride, anhydrotetracycline hydrochloride, and chlortetracycline hydrochloride obtained with solution A are not more intense than those obtained with solution C (0.5% of 4-epianhydrotetracycline hydrochloride), solution E (0.5% of anhydrotetracycline hydrochloride), and solution F (2% of chlortetracycline hydrochloride). The test is not valid unless the chromatogram obtained with solution G shows 5 clearly separated spots.

Anhydroderivatives. Dissolve about 0.2 g, accurately weighed, in sufficient hydrochloric acid (0.02 mol/l) VS to produce 50 ml. Place 10.0 ml in a separator, add 10 ml of chloroform R and 10 ml of citrate buffer, pH 5.4 TS, and shake for 2 minutes. Separate the chloroform layer and measure the absorbance at 437 nm against a solvent cell containing chloroform R; not more than 0.18 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).

Assay

A. Dissolve about 0.25 g, accurately weighed and previously dried at 60 °C under reduced pressure, in 5 ml of formic acid (~1080 g/l) TS and 10 ml of glacial acetic acid R1, add 10 ml of dioxan R, 5 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 48.09 mg of $C_{22}H_{24}N_2O_8$,HCl.

B. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5–6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of tetracycline (usually between 2 and 20 IU), and an incubation temperature of 37–39 °C, or (*b*) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9–6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of tetracycline (usually between 0.5 and 2 IU), and an incubation temperature of 30–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 950 IU per mg, calculated with reference to the dried substance.

Additional requirements for Tetracycline hydrochloride for sterile use

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.

Sterility. Complies with the 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure. Use the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

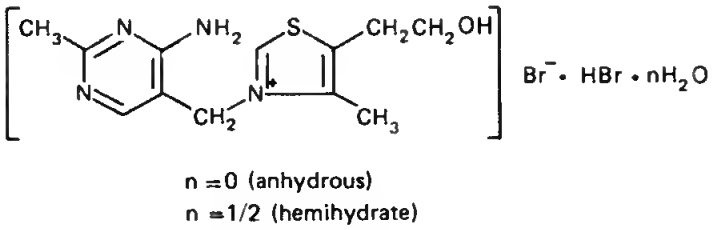
THIAMINI HYDROBROMIDUM
THIAMINE HYDROBROMIDE

Thiamine hydrobromide, anhydrous
Thiamine hydrobromide hemihydrate

Molecular formula. $C_{12}H_{17}BrN_4OS,HBr$ (anhydrous); $C_{12}H_{17}BrN_4OS,HBr,\frac{1}{2}H_2O$ (hemihydrate).

Relative molecular mass. 426.2 (anhydrous); 435.2 (hemihydrate).

Graphic formula.



n = 0 (anhydrous)
n = 1/2 (hemihydrate)

Chemical name. Thiamine bromide, monohydrobromide; 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium bromide, monohydrobromide; CAS Reg. No. 4234-86-0 (anhydrous).

Thiamine bromide, monohydrobromide, hemihydrate; 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium bromide, mono-hydrobromide, hemihydrate; CAS Reg. No. 62084-87-1 (hemihydrate).

Description. A white to yellowish white, crystalline powder; odour, slight and characteristic.

Solubility. Freely soluble in water and in methanol R; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Component of vitamin B.

Storage. Thiamine hydrobromide should be kept in a tightly closed, non-metallic container, protected from light.

Requirements

Definition. Thiamine hydrobromide contains not less than 98.0% and not more than 101.0% of $C_{12}H_{17}BrN_4OS,HBr$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS, 0.5 ml of potassium ferricyanide (10 g/l) TS; the solution remains pale yellow. Shake with 5 ml of 2-butanol R and allow to stand for 5–10 minutes; in bright daylight or in ultraviolet light (365 nm) the 2-butanol layer shows a blue fluorescence.

B. Spread a small quantity of the powder on a watch-glass; the odour is slight and characteristic, resembling that of yeast.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of bromides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.6 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C. Anhydrous thiamine hydrobromide loses not more than 5.0 mg/g. Thiamine hydrobromide hemihydrate loses not more than 25 mg/g.

pH value. pH of a 0.06 g/ml solution, 2.7–3.4.

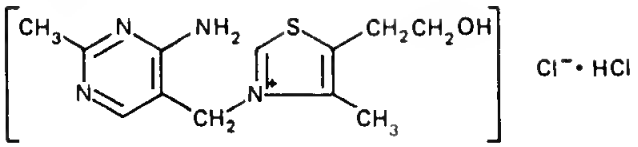
Assay. Dissolve about 0.30 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 21.31 mg of $C_{12}H_{17}BrN_4OS,HBr$.

THIAMINI HYDROCHLORIDUM
THIAMINE HYDROCHLORIDE

Molecular formula. $C_{12}H_{17}ClN_4OS,HCl$

Relative molecular mass. 337.3

Graphic formula.



Chemical name. Thiamine chloride, hydrochloride; 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride, monohydrochloride; CAS Reg. No. 67-03-8.

Description. Colourless crystals or a white or yellowish white, crystalline powder; odour, slight and characteristic.

Solubility. Soluble in 1 part of water and in 100 parts of ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

Category. Component of vitamin B.

Storage. Thiamine hydrochloride should be kept in a tightly closed, non-metallic container, protected from light.

Additional information. Even in the absence of light, Thiamine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. When exposed to air, the anhydrous product rapidly absorbs about 4 g of water per 100 g. Melting temperature, about 248 °C with some decomposition. In solution at pH 4.0 or less, it loses its activity only very slowly. Neutral and alkaline solutions deteriorate rapidly, especially in contact with air.

Requirements

Definition. Thiamine hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{12}H_{17}ClN_4OS,HCl$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS and 0.5 ml of potassium ferricyanide (10 g/l) TS; the solution remains pale yellow.

   Shake with 5 ml of 2-butanol R and allow to stand for 5–10 minutes; in bright daylight or in ultraviolet light (365 nm) the 2-butanol layer shows a blue fluorescence.

B. Spread a small quantity of the powder on a watch-glass; the odour is slight and characteristic, resembling that of yeast.

C. A 0.05 g/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 2.0 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 50 mg/g.

pH value. pH of a 25 mg/ml solution, 2.7–3.3.

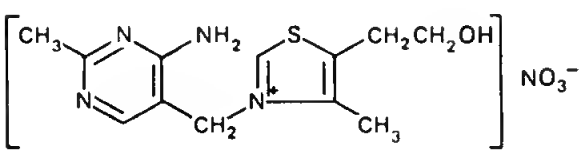
Assay. Dissolve about 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 16.86 mg of $C_{12}H_{17}ClN_4OS,HCl$.

THIAMINI MONONITRAS
THIAMINE MONONITRATE

Molecular formula. $C_{12}H_{17}N_5O_4S$

Relative molecular mass. 327.4

Graphic formula.



Chemical name. Thiamine nitrate (salt); 3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate (salt); CAS Reg. No. 532-43-4.

Description. Colourless crystals or a white, crystalline powder; odour, slight and characteristic.

Solubility. Sparingly soluble in water; very slightly soluble in ethanol (~750 g/l) TS.

Category. Component of vitamin B.

Storage. Thiamine mononitrate should be kept in a tightly closed, non-metallic container, protected from light.

Additional information. Even in the absence of light, Thiamine mononitrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Thiamine mononitrate contains not less than 98.0% and not more than 101.0% of $C_{12}H_{17}N_5O_4S$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS and 0.5 ml of potassium ferricyanide (10 g/l) TS; the solution remains pale yellow. Shake with 5 ml of 2-butanol R and allow to stand for 5–10 minutes; in bright daylight or in ultraviolet light (365 nm) the 2-butanol layer shows a blue fluorescence.

B. Spread a small quantity of the powder on a watch-glass; the odour is slight and characteristic, resembling that of yeast.

C. To 2 ml of a 0.05 g/ml solution add 2 ml of ferrous sulfate (15 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of nitrates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 0.2 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 20 mg/ml solution, 6.0–7.5.

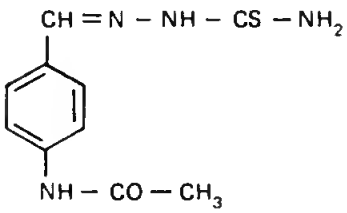
Assay. Dissolve about 0.1 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 0.15 ml of l-naphtholbenzein/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS to a green end-point, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 16.37 mg of $C_{12}H_{17}N_5O_4S$.

THIOACETAZONUM
THIOACETAZONE

Molecular formula. $C_{10}H_{12}N_4OS$

Relative molecular mass. 236.3

Graphic formula.



Chemical name. 4′-Formylacetanilide 4′-(thiosemicarbazone); *N*-[4-[[(aminothioxomethyl)hydrazono]methyl]phenyl]acetamide; CAS Reg. No. 104-06-3.

Description. Pale yellow crystals or a yellow, crystalline powder; almost odourless.

Solubility. Very slightly soluble in water; slightly soluble in ethanol (~750 g/l) TS and methanol R; soluble in 10 parts of dimethylformamide R.

Category. Antituberculosis drug.

Storage. Thioacetazone should be kept in a tightly closed container, protected from light.

Requirements

Definition. Thioacetazone contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}N_4OS$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from thioacetazone RS or with the *reference spectrum* of thioacetazone.

B. The absorption spectrum of a 3.0 µg/ml solution in dehydrated ethanol R, when observed between 230 nm and 350 nm, exhibits a maximum at about 328 nm; the absorbance of a 1-cm layer at this wavelength is about 0.58.

C. Dissolve 10 mg by heating in 1 ml of sodium hydroxide (5 mol/l) VS, add 0.25 ml of lead acetate (80 g/l) TS and boil for 1 minute; a black precipitate is produced.

D. About 10 mg yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a red colour.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash. Not more than 2.0 mg/g.

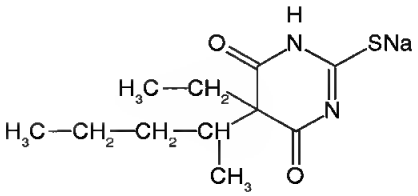
Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 9 volumes of methanol R and 1 volume of water containing (A) 2.0 mg of the test substance per ml, and (B) 4.0 µg of *p*-acetamidobenzalazine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray it evenly with nitric acid (~130 g/l) TS, and examine the chromatogram in ultraviolet light (365 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Thiosemicarbazide. Finely powder the test substance and to about 2 g, accurately weighed, add sufficient water to produce 50 ml. Allow to stand for at least 1 hour, shaking occasionally. Filter, reject the first few ml of the filtrate, and transfer 25 ml of the filtrate to a 250-ml conical flask. Acidify with sulfuric acid (~100 g/l) TS and titrate with ceric sulfate (0.1 mol/l) VS, using *o*-phenanthroline TS as indicator, to a blue end-point that persists for 1 minute. Not more than 0.8 ml of ceric sulfate (0.1 mol/l) VS is required (not more than 1.0 mg/g).

Assay. Dissolve about 0.1 g, accurately weighed, in 60 ml of methanol R by warming to 60 °C on a water-bath. Slowly add 20 ml of hot silver nitrate/methanol TS, maintain the temperature at 60 °C until the formed precipitate congeals and settles, leaving a clear supernatant liquor. Cool and filter through a dried and weighed sintered-glass crucible and wash the precipitate with methanol R until the washings are free from silver. Dry to constant weight at 105 °C, cool and weigh. Each g of precipitate is equivalent to 460.6 mg of $C_{10}H_{12}N_4OS$.

THIOPENTALUM NATRICUM
THIOPENTAL SODIUM



$C_{11}H_{17}N_2NaO_2S$

Relative molecular mass. 264.3

Chemical name. Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate; 5-ethyldihydro-5-(1-methylbutyl)-2-thioxo-4,6(1*H*,5*H*)-pyrimidinedione monosodium salt; CAS Reg. No. 71-73-8.

Description. A yellowish white powder; odour, characteristic.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. General anaesthetic.

Storage. Thiopental sodium should be kept in a tightly closed container, protected from light.

Additional information. Thiopental sodium is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Thiopental sodium contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{11}H_{17}N_2NaO_2S$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Place about 0.5 g in a separatory funnel, add 10 ml of water and acidify with hydrochloric acid (~70 g/l) TS. Shake with 20 ml of ether R, separate the ether layer, wash with 10 ml of water, dry over anhydrous sodium sulfate R, and filter. Evaporate the filtrate to dryness over a water-bath and dry the residue at 100–105 °C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from thiopental RS or with the *reference spectrum* of thiopental.

B. See the test described below under "Related substances". Apply 10 µl of each of solutions B and C to the plate and develop the chromatogram for a distance of 18 cm. The principal spot obtained with solution B corresponds in position and intensity with that obtained with solution C.

C. Fuse 0.2 g with 1 g of sodium hydroxide R in a test-tube until the glass glows red; the melt turns red-brown and vapours are evolved. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range. Cool and add 5 ml of water to the melt, mix well, and filter. Acidify the filtrate with sulfuric acid (~100 g/l) TS and heat gently; the vapours evolved turn a strip of lead nitrate paper R to brown and then to black.

D. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Clarity and colour of solution. A solution of 1 g in 10 ml of water is clear and not more intensely coloured than standard colour solution Gn5 when compared as described under 1.11 Colour of liquids.

Loss on drying. Dry at 80 °C for 4 hours; it loses not more than 20 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and the lower layer of a mixture of 5 volumes of ammonia (~260 g/l) TS, 15 volumes of ethanol (~750 g/l) TS, and 80 volumes of chloroform R as the mobile phase. Apply separately to the plate 20 µl of each of three solutions containing (A) 10 mg of Thiopental sodium per ml (disregard any slight residue), for solution (B) dilute 1 ml of solution A to 10 ml, for solution (C) dissolve 85 mg of thiopental RS in 10 ml of sodium hydroxide (~80 g/l) TS and dilute with sufficient water to produce 100 ml, and for solution (D) dilute 0.5 ml of solution A to 100 ml. After removing the plate from the chromatographic chamber, examine the chromatogram immediately in ultraviolet light.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D (0.5%). Disregard any spot at the point of application.

Assay. Dissolve about 0.15 g, accurately weighed, in 5 ml of water, add 2 ml of sulfuric acid (~100 g/l) TS, and extract with four 10-ml quantities of chloroform R. Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water-bath, and dissolve the residue in 30 ml of dimethylformamide R previously neutralized with lithium methoxide (0.1 mol/l) VS. Titrate immediately with lithium methoxide (0.1 mol/l) VS, using 0.1 ml of thymol blue/methanol TS as indicator, until a blue colour is obtained. Protect the solution from atmospheric carbon dioxide during the titration.

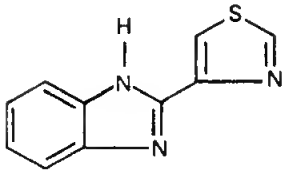
Each ml of lithium methoxide (0.1 mol/l) VS is equivalent to 26.43 mg of $C_{11}H_{17}N_2NaO_2S$.

TIABENDAZOLUM
TIABENDAZOLE

Molecular formula. $C_{10}H_7N_3S$

Relative molecular mass. 201.3

Graphic formula.



Chemical name. 2-(4-Thiazolyl)benzimidazole; 2-(4-thiazolyl)-1*H*-benzimidazole; CAS Reg. No. 148-79-8.

Description. A white to almost white powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in 150 parts of ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Anthelmintic.

Storage. Tiabendazole should be kept in a well-closed container.

Requirements

Definition. Tiabendazole contains not less than 98.0% and not more than 101.0% of $C_{10}H_7N_3S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from tiabendazole RS or with the *reference spectrum* of tiabendazole.

B. The absorption spectrum of a 4.0 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits maxima at about 243 nm and 302 nm; the absorbances of a 1-cm layer at these maxima are about 0.23 and 0.49, respectively.

C. Dissolve 5 mg in 5 ml of hydrochloric acid (0.1 mol/l) VS, add 3 mg of 1,4-phenylenediamine dihydrochloride R, and shake until dissolved. Add 0.1 g of zinc R powder, mix, allow to stand for 2 minutes, and add 10 ml of ferric ammonium sulfate (45 g/l) TS; a deep blue or blue-violet colour is produced.

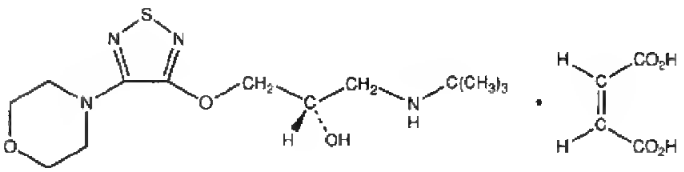
Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 50 volumes of toluene R, 20 volumes of glacial acetic acid R, 8 volumes of acetone R, and 2 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per ml and (B) 0.15 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.16 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.13 g of $C_{10}H_7N_3S$.

TIMOLOLI MALEAS
TIMOLOL MALEATE



$C_{13}H_{24}N_4O_3S,C_4H_4O_4$

Relative molecular mass. 432.5

Chemical name. (–)-(*S*)-1-(*tert*-Butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (1 : 1) (salt); (*S*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (*Z*)-2-butenedioate (1 : 1) (salt); CAS Reg. No. 26921-17-5.

Description. A white or almost white powder; odourless or almost odourless.

Solubility. Soluble in water, methanol R, and ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Antiglaucoma drug.

Storage. Timolol maleate should be kept in a well-closed container, protected from light.

Requirements

Timolol maleate contains not less than **98.0%** and not more than the equivalent of **101.0%** of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from timolol maleate RS or with the *reference spectrum* of timolol maleate.

B. The absorption spectrum of a 25 µg/ml solution in sulfuric acid (0.05 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 295 nm; the absorbance of a 1-cm layer at this wavelength is about 0.52.

C. Dissolve 0.2 g in 3 ml of water, add 2 ml of sodium hydroxide (~200 g/l) TS, and shake with three quantities, each of 3 ml, of ether R. Warm the aqueous layer in a water-bath for 10 minutes, add 2 ml of bromine TS1, boil, and cool. Add 0.2 ml of this solution to 10 mg of resorcinol R dissolved in 3 ml of sulfuric acid (~1760 g/l) TS, and heat in a water-bath for 15 minutes; a bluish black colour is produced.

Specific optical rotation. Use a 50 mg/ml solution in hydrochloric acid (1 mol/l) VS; $[\alpha]_D^{20\,°C} = -11.7\,°$ to $-12.5\,°$.

Clarity and colour of solution. A solution of 0.2 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 100 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury); it loses not more than 5.0 mg/g.

pH value. pH of a 20 mg/ml solution, 3.8–4.3.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 80 volumes of

dichloromethane R, 20 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of three solutions in methanol R containing (A) 50 mg of Timolol maleate per ml, (B) 0.2 mg of Timolol maleate per ml, and (C) 0.1 mg of Timolol maleate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Then expose the plate to iodine vapours for 2 hours and examine the chromatogram in daylight.

Using both methods of visualization, any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B, and not more than two such spots are more intense than that obtained with solution C.

Assay. Dissolve about 0.85 g, accurately weighed, in 90 ml of glacial acetic acid R1, add 3 drops of 1-naphtholbenzein/acetic acid TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 43.25 mg of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$.

Additional requirement for Timolol maleate for sterile use

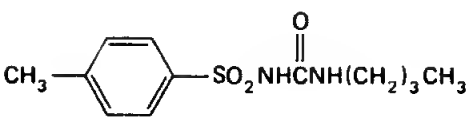
Complies with 3.2.1 Test for sterility of non-injectable preparations.

TOLBUTAMIDUM
TOLBUTAMIDE

Molecular formula. $C_{12}H_{18}N_2O_3S$

Relative molecular mass. 270.4

Graphic formula.



Chemical name. 1-Butyl-3-(*p*-tolylsulfonyl)urea; *N*-[(butylamino)carbonyl]-4-methylbenzenesulfonamide; CAS Reg. No. 64-77-7.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Practically insoluble in water; soluble in 10 parts of ethanol (~750 g/l) TS; soluble in acetone R.

Category. Antidiabetic.

Storage. Tolbutamide should be kept in a tightly closed container, protected from light.

Additional information. Even in the absence of light, Tolbutamide is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Tolbutamide contains not less than 99.0% and not more than 101.0% of $C_{12}H_{18}N_2O_3S$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from tolbutamide RS or with the *reference spectrum* of tolbutamide.

B. Boil 0.2 g with 8 ml of sulfuric acid (~700 g/l) TS under a reflux condenser for 30 minutes. Cool and filter. Keep the precipitate for test C. Make the filtrate strongly alkaline by adding sodium hydroxide (~300 g/l) TS, and carry out a steam distillation for 30 minutes. Collect the distillate in 30 ml of hydrochloric acid (0.1 mol/l) VS. To 2 ml of the solution containing the distillate add 0.2 g of sodium acetate R and 10 ml of sodium tetraborate (10 g/l) TS. Cool the mixture for 10 minutes in an ice-bath, add 1 ml of 4-nitroaniline TS1 and 2.7 ml of sodium nitrite (100 g/l) TS and allow to stand for 30 minutes. Add 2.5 ml of sodium hydroxide (~80 g/l) TS drop by drop; after a few minutes an intense red colour develops.

C. Wash the precipitate obtained in test B with 4 ml of water and dry at 105 °C; melting temperature, about 136 °C.

Melting range. 126–130 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 15 volumes of 2-propanol R, 3 volumes of cyclohexane R, 1 volume of ammonia (~260 g/l) TS and 1 volume of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in acetone R containing (A) 10 mg of the test substance per ml and (B) 0.050 mg of 4-toluenesulfonamide R per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, heat at 110 °C for 10 minutes, spray the hot plate with sodium hypochlorite TS1 and dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with 1 drop of potassium iodide/starch TS; avoid prolonged exposure to cold air. Spray the plate with potassium iodide/starch TS, allow it to stand for 5 minutes, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

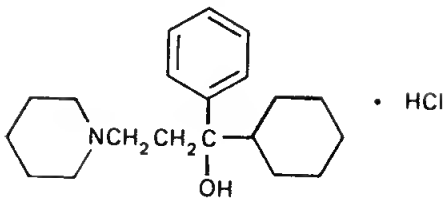
Assay. Dissolve about 0.55 g, accurately weighed, in 30 ml of ethanol (~750 g/l) TS previously neutralized to phenolphthalein/ethanol TS, add 20 ml of carbon-dioxide-free water R, and titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is equivalent to 27.04 mg of $C_{12}H_{18}N_2O_3S$.

TRIHEXYPHENIDYLI HYDROCHLORIDUM
TRIHEXYPHENIDYL HYDROCHLORIDE

Molecular formula. $C_{20}H_{31}NO,HCl$

Relative molecular mass. 337.9

Graphic formula.



Chemical name. α-Cyclohexyl-α-phenyl-1-piperidinepropanol hydrochloride; CAS Reg. No. 52-49-3.

Other names. Benzhexol hydrochloride; cyclodolum.

Description. A white or off-white, crystalline powder; odourless or almost odourless.

Solubility. Slightly soluble in water; soluble in ethanol (~750 g/l) TS, and methanol R.

Category. Anticholinergic; antiparkinsonism drug.

Storage. Trihexyphenidyl hydrochloride should be kept in a tightly closed container.

Requirements

Definition. Trihexyphenidyl hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{20}H_{31}NO,HCl$, calculated with reference to the dried substance.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from trihexyphenidyl hydrochloride RS or with the *reference spectrum* of trihexyphenidyl hydrochloride.

B. Dissolve 0.5 g in 5 ml of warm methanol R and add sufficient sodium hydroxide (~80 g/l) TS to make the solution alkaline to litmus paper R; a white precipitate is formed. Collect the precipitate, wash with a small portion of water, recrystallize from methanol R, and dry under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R for 2 hours; melting temperature, about 115 °C (trihexyphenidyl base).

C. A 0.05 g/ml solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. Dissolve 1.0 g in 100 ml of carbon-dioxide-free water R by warming, then cool; pH of this solution, 5.0–6.0.

Piperidylpropiophenone. Dissolve 0.10 g in a mixture of 40 ml of water and 1 ml of hydrochloric acid (1 mol/l) VS with the aid of heat, cool, and add sufficient water to produce 100 ml. Measure the absorbance of a 1-cm layer at the maximum at about 247 nm; not more than 0.5.

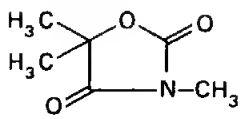
Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.79 mg of $C_{20}H_{31}NO,HCl$.

TRIMETHADIONUM
TRIMETHADIONE

Molecular formula. $C_6H_9NO_3$

Relative molecular mass. 143.1

Graphic formula.



Chemical name. 3,5,5-Trimethyl-2,4-oxazolidinedione; CAS Reg. No. 127-48-0.

Description. Colourless, granular crystals; odour, slightly camphoraceous.

Solubility. Soluble in water; freely soluble in ethanol (~750 g/l) TS, and ether R.

Category. Anticonvulsant.

Storage. Trimethadione should be kept in a well-closed container, and stored in a cool place.

Requirements

Definition. Trimethadione contains not less than 98.0% and not more than 101.0% of $C_6H_9NO_3$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from trimethadione RS or with the *reference spectrum* of trimethadione.

B. To 5 ml of a 20 mg/ml solution add 2 ml of barium hydroxide (15 g/l) TS; a precipitate is immediately produced.

C. Heat 0.5 g with 10 ml of sodium hydroxide (~80 g/l) TS on a water-bath for 30 minutes, evaporate to low bulk, cool in ice, and cautiously add hydrochloric acid (~70 g/l) TS until acid to litmus paper R. To 0.5 ml add 2 drops of ferric chloride (25 g/l) TS; a deep yellow colour is produced. Retain the remainder of the solution for test D.

D. Extract the remainder of the solution obtained in test C 3 times with ether R, using 10 ml each time; evaporate the combined ether extracts on a water-bath for 30 minutes and scratch the inner surface of the container to induce crystallization; melting temperature, about 80 °C (α-hydroxyisobutyric acid).

Melting range. 45–47 °C, determined without previous drying.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight over silica gel, desiccant, R at ambient temperature; it loses not more than 5.0 mg/g.

Assay. Carry out the assay as described under 1.14.5 Gas chromatography. As an internal standard use 2-phenylethanol TS. Use the following 3 solutions: (1) to 0.10 g of trimethadione RS add 5 ml of 2-phenylethanol TS and sufficient methanol R to produce 10 ml, (2) dissolve 0.20 g of the substance being examined in sufficient methanol R to produce 10 ml, and (3) to 0.20 g of the substance being examined add 5 ml of 2-phenylethanol TS and sufficient methanol R to produce 10 ml. For the procedure use a glass column 1.5 m long

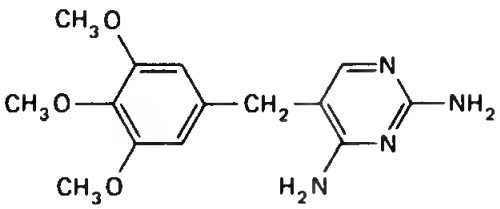
and 0.4 cm in internal diameter packed with an adequate quantity of an adsorbent composed of 10 g of diethylene glycol succinate R supported on 90 g of acid-washed, silanized kieselguhr R4. Maintain the column at 105 °C, use nitrogen R as the carrier gas and a flame ionization detector. Prepare chromatograms A, B, and C from solutions 1, 2 and 3, respectively. Measure the appropriate peak areas in chromatograms A, B, and C, and calculate the content of $C_6H_9NO_3$, using the data obtained from chromatograms A and C, introducing if necessary the correction resulting from chromatogram B.

TRIMETHOPRIMUM
TRIMETHOPRIM

Molecular formula. $C_{14}H_{18}N_4O_3$

Relative molecular mass. 290.3

Graphic formula.



Chemical name. 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine; 5-[(3,4,5-trimethoxyphenyl)methyl]-2,4-pyrimidinediamine; CAS Reg. No. 738-70-5.

Description. A white, crystalline powder; odourless or almost odourless.

Solubility. Sparingly soluble in water; soluble in methanol R; practically insoluble in ether R.

Category. Antibacterial.

Storage. Trimethoprim should be kept in a well-closed container.

Requirements

Definition. Trimethoprim contains not less than 98.5% and not more than 101.0% of $C_{14}H_{18}N_4O_3$, calculated with reference to the dried substance.

Identity tests

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from trimethoprim RS or with the *reference spectrum* of trimethoprim.

B. Dissolve 25 mg in 5 ml of sulfuric acid (0.005 mol/l) VS, heat if necessary, and add 2 ml of a mixture of 1.6 g of potassium permanganate R dissolved in sufficient sodium hydroxide (0.1 mol/l) VS to produce 100 ml. Heat to boiling and add to the hot solution 0.4 ml of formaldehyde TS. Mix, add 1 ml of sulfuric acid (0.5 mol/l) VS, mix, and again heat to boiling. Cool to room temperature and filter. To the filtrate add 2 ml of chloroform R and shake the flask vigorously; a green fluorescence is produced in the chloroform layer when examined in ultraviolet light (365 nm).

C. Melting temperature, about 200 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. Shake 0.20 g with 20 ml of carbon-dioxide-free water R for 1 minute and filter; pH of the filtrate, 7.5–8.5.

Related substances

- The operations must be performed in a well-ventilated hood.

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 85 volumes of ethyl acetate R, 10 volumes of methanol R, 5 volumes of water, and 2 volumes of anhydrous formic acid R as the mobile phase; an unlined chromatographic chamber should be used and the solvent front allowed to ascend 17 cm above the line of application. Apply separately to the plate 5 µl of each of 2 solutions in a mixture of 5 volumes of chloroform R, 4.5 volumes of methanol R, and 1 volume of water containing (A) 40 mg of the test substance per ml and (B) 0.080 mg of the test substance per ml. Pour the mobile phase into the chamber and insert the plate immediately so as to avoid prior saturation of the chamber. After removing the plate from the chromatographic chamber, allow it to dry in a stream of cold air for 5 minutes, and examine the chromatogram in ultraviolet light (254 nm). Place the plate in a closed chamber containing chlorine, produced by mixing equal volumes of a 15 mg/ml solution of potassium permanganate R and hydrochloric acid (~70 g/l) TS, placed at the bottom of the chamber, and allow to stand for 20 minutes. Remove the plate from the chamber and drive off the chlorine in a current of cold air until the area below the line of application does not give any blue colour on the addition of 0.05 ml

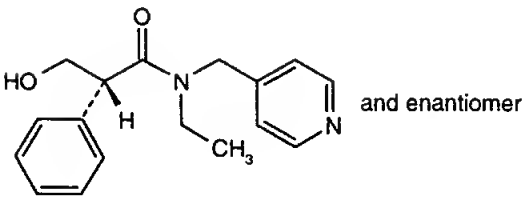
of starch/iodide TS. Spray the plate with starch/iodide TS, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.6 g, accurately weighed, in 30 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration. Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 29.03 mg of $C_{14}H_{18}N_4O_3$.

Additional requirement for Trimethoprim for parenteral use

Complies with the monograph for "Parenteral preparations".

TROPICAMIDUM
TROPICAMIDE



$C_{17}H_{20}N_2O_2$

Relative molecular mass. 284.4

Chemical name. *N*-Ethyl-2-phenyl-*N*-(4-pyridylmethyl)hydracrylamide; *N*-ethyl-β-(hydroxymethyl)-*N*-(4-pyridinylmethyl)-benzeneacetamide; CAS Reg. No. 1508-75-4.

Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water; freely soluble in dichloromethane R and ethanol (~750 g/l) TS.

Category. Mydriatic.

Storage. Tropicamide should be kept in a tightly closed container, protected from light.

Labelling. The designation Tropicamide for sterile non-injectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

Requirements

Tropicamide contains not less than **99.0%** and not more than **101.0%** of $C_{17}H_{20}N_2O_2$, calculated with reference to the dried substance.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from tropicamide RS or with the *reference spectrum* of tropicamide.

B. The absorption spectrum of a 0.04 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 254 nm; the absorbance of a 1-cm layer at this wavelength is about 0.72.

C. Dissolve 5 mg in 3 ml of a mixture of 9 ml of acetic anhydride R, 1 ml of acetic acid (~300 g/l) TS, and 0.10 g of citric acid R. Heat on a water-bath for 5–10 minutes: a reddish yellow colour is produced.

D. Melting temperature, about 97 °C.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 5.0 mg/g.

Tropic acid. To 10.0 mg add 5 mg of sodium tetraborate R and 0.35 ml of a freshly prepared solution containing 1.0 g of dimethylaminobenzaldehyde R in 10 ml of a mixture of 9 volumes of sulfuric acid (~1760 g/l) TS and 1 volume of water. Heat on a water-bath for 3 minutes. Cool in ice-water and add 5 ml of acetic anhydride R; no violet-red colour develops (0.05%).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 95 volumes of dichloromethane R, 5 volumes of methanol R, and 0.5 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in dichloromethane R containing (A) 20 mg of Tropicamide per ml, (B) 0.10 mg of Tropicamide per ml, and (C) 40 µg of Tropicamide per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Not more than one such spot is more intense than that obtained with solution C (0.2%).

Assay. Dissolve about 0.2 g, accurately weighed, in 50 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, using 1-naphtholbenzein/acetic acid TS as indicator until the colour changes from orange to green as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 28.44 mg of $C_{17}H_{20}N_2O_2$.

Additional requirement for sterile non-injectable Tropicamide

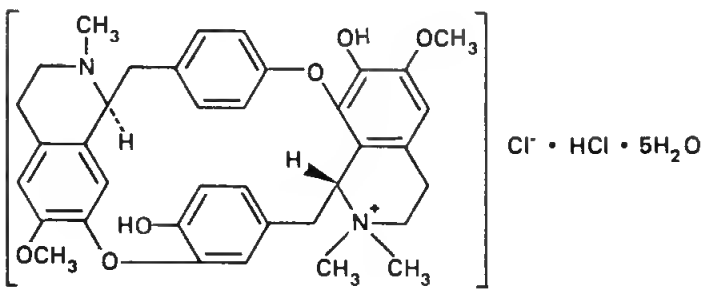
Complies with 3.2.1 Test for sterility of non-injectable preparations.

TUBOCURARINI CHLORIDUM
TUBOCURARINE CHLORIDE

Molecular formula. $C_{37}H_{41}ClN_2O_6,HCl,5H_2O$

Relative molecular mass. 771.7

Graphic formula.



Chemical name. (+)-Tubocurarine chloride hydrochloride, pentahydrate; 7′,12′-dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethyltubocuraranium chloride hydrochloride pentahydrate; CAS Reg. No. 6989-98-6 (pentahydrate).

Description. A white to yellowish white, crystalline powder; odourless.

Solubility. Soluble in 20 parts of water and 30 parts of ethanol (~750 g/l) TS; practically insoluble in acetone R, and ether R.

Category. Muscle relaxant.

Storage. Tubocurarine chloride should be kept in a tightly closed container.

Additional information. Tubocurarine chloride melts at about 270 °C with decomposition.

Requirements

Definition. Tubocurarine chloride contains not less than 98.0% and not more than 102.0% of $C_{37}H_{41}ClN_2O_6,HCl$, calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water and add 1 ml of mercuric nitrate TS; a cherry red colour is slowly produced.

B. Dissolve 10 mg in 1 ml of water and add 0.1 ml of ferric chloride (25 g/l) TS; a green colour is produced, which becomes brown on warming on a water-bath.

C. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Specific optical rotation. Use a 10 mg/ml solution, which has been allowed to stand for 3 hours, and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = +210$ to $+220°$.

Chloroform-soluble substances. Dissolve 0.25 g in 150 ml of water, add 5 ml of a saturated solution of sodium hydrogen carbonate R, and extract with 3 quantities, each of 20 ml, of chloroform R. Wash the combined chloroform extracts with 10 ml of water, filter the chloroform solution into a beaker, wash the filter with 2 successive quantities, each of 5 ml, of chloroform R, and add the washings to the filtrate. Evaporate the combined filtrate and washings on a water-bath and dry the residue at 105 °C for 1 hour; the weight of the residue is not more than 5 mg (2.0%). Add 10 ml of water to the residue; the residue does not dissolve. Then add 1 ml of hydrochloric acid (~70 g/l) TS; the residue dissolves.

Sulfated ash. Not more than 2.5 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not less than 90 mg/g and not more than 120 mg/g.

pH value. pH of a 10 mg/ml solution, 4.0–6.0.

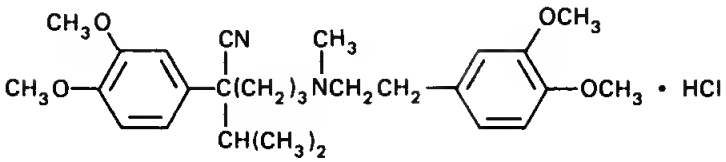
Assay. Dissolve about 0.5 g, accurately weighed, in 20 ml of glacial acetic acid R1 by warming on a water-bath, cool, and add 60 ml of acetic anhydride R and 10 ml of mercuric acetate/acetic acid TS. Titrate with perchloric acid (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 34.08 mg of $C_{37}H_{41}ClN_2O_6$,HCl.

VERAPAMILI HYDROCHLORIDUM
VERAPAMIL HYDROCHLORIDE

Molecular formula. $C_{27}H_{38}N_2O_4$,HCl

Relative molecular mass. 491.1

Graphic formula.



Chemical name. 5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride; α-[3-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)benzeneacetonitrile monohydrochloride; CAS Reg. No. 152-11-4.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Solubility. Soluble in 20 parts of water; sparingly soluble in ethanol (~750 g/l) TS.

Category. Antianginal drug.

Storage. Verapamil hydrochloride should be kept in a well-closed container, protected from light.

Requirements

Definition. Verapamil hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{27}H_{38}N_2O_4$,HCl, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, D and E may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from verapamil hydrochloride RS or with the *reference spectrum* of verapamil hydrochloride.

B. The absorption spectrum of a 20 µg/ml solution in hydrochloric acid (0.01 mol/l) VS, when observed between 220 nm and 350 nm, exhibits maxima at about 229 nm and 278 nm; the absorbances of a 1-cm layer at these maxima are about 0.63 and 0.24, respectively.

C. Dissolve 20 mg in 2.5 ml of water, add 0.5 ml of sulfuric acid (~570 g/l) TS and 0.2 ml of potassium permanganate (10 g/l) TS; a violet precipitate is produced, which quickly dissolves to produce a very pale yellow solution.

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

E. Melting temperature, about 143 °C.

Clarity and colour of solution. A solution of 0.50 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

Readily carbonizable substances. Dissolve 0.10 g in 5 ml of sulfuric acid (~1760 g/l) TS. After 5 minutes the solution is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under 1.11 Colour of liquids.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 4.5–6.5.

Related substances

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance (a precoated plate from a com-

mercial source is suitable) and a mixture of 85 volumes of cyclohexane R and 15 volumes of diethylamine R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in chloroform R containing (A) 50 mg of the test substance per ml, (B) 25 µg of the test substance per ml, and (C) 50 µg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry at room temperature for 10 minutes and develop the plate a second time. Remove the plate, heat it at 110 °C for 1 hour, allow it to cool, and spray it with a solution prepared by dissolving 5 g of ferric chloride R and 2 g of iodine R in 50 ml of tartaric acid (200 g/l) TS, applying a total of 15–20 ml of the reagent. Examine the plate immediately in daylight disregarding any spot on the line of application. Up to 3 secondary spots may be more intense than the spot obtained with solution B but they must be less intense than the spot obtained with solution C. Any other secondary spots in the chromatogram obtained with solution A must be less intense than the spot obtained with solution B.

B. Carry out test A once more using a mixture of 70 volumes of toluene R, 20 volumes of methanol R, 5 volumes of acetone R, and 5 volumes of glacial acetic acid R as the mobile phase. The result is the same as that with test A.

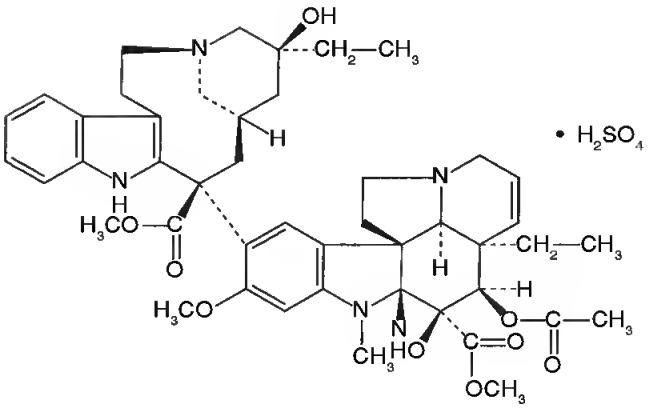
Assay. Dissolve about 0.5 g, accurately weighed, in 30 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS followed by 0.15 ml of 1-naphtholbenzein/acetic acid TS as indicator, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 49.11 mg of $C_{27}H_{38}N_2O_4$,HCl.

Additional requirements for Verapamil hydrochloride for parenteral use

Complies with the monograph for "Parenteral preparations".

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 16.7 IU of endotoxin RS per mg.

VINBLASTINI SULFAS
VINBLASTINE SULFATE



$C_{46}H_{58}N_4O_9,H_2SO_4$

Relative molecular mass. 909.1

Chemical name. Vincaleukoblastine sulfate (1:1) (salt); CAS Reg. No. 143-67-9.

Description. A white to slightly yellow, amorphous or crystalline powder.

Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Vinblastine sulfate should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Additional information. *CAUTION:* Vinblastine sulfate must be handled with care, avoiding contact with the skin and inhalation of airborne particles. It is very hygroscopic and unstable. Before the bottle is opened, it should be allowed to come to room temperature in a desiccator.

Requirements

Vinblastine sulfate contains not less than **96.0%** and not more than the equivalent of **101.0%** of $C_{46}H_{58}N_4O_9,H_2SO_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from vinblastine sulfate RS or with the *reference spectrum* of vinblastine sulfate.

B. See the test described below under "Related alkaloids". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution C.

C. To 1 mg add 0.2 ml of vanillin/hydrochloric acid TS and allow to stand for 1 minute; a pink colour is produced (distinction from vincristine sulfate).

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Specific optical rotation. Use a 20 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C} = -20$ to $-35\,°$.

Clarity of solution. A solution of 30 mg in 10 ml of water is clear.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 16 hours; it loses not more than 170 mg/g.

pH value. pH of a 1.5 mg/ml solution, 3.5–5.0.

Related alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 80 volumes of toluene R, 40 volumes of chloroform R, and 6 volumes of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in methanol R containing (A) 10 mg of Vinblastine sulfate per ml, (B) 0.2 mg of vincristine sulfate RS per ml, and (C) 10 mg of vinblastine sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 10 mg, accurately weighed, in sufficient methanol R to produce 500 ml.

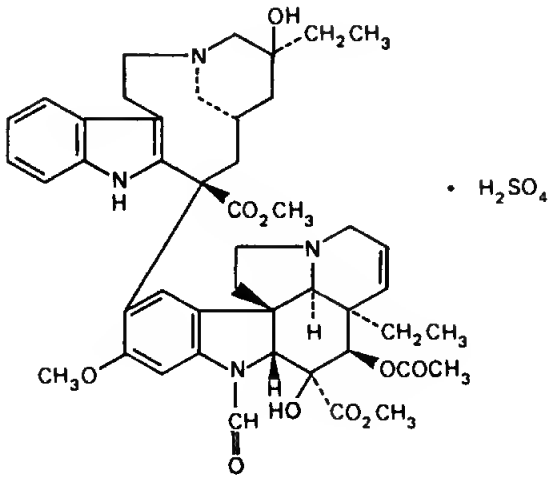
Measure the absorbance of this solution in a 1-cm layer at the maximum at about 267 nm. Calculate the content of $C_{46}H_{58}N_4O_9,H_2SO_4$, using the absorptivity value of 18.5 ($A^{1\%}_{1\,cm} = 185$).

VINCRISTINI SULFAS
VINCRISTINE SULFATE

Molecular formula. $C_{46}H_{56}N_4O_{10},H_2SO_4$

Relative molecular mass. 923.0

Graphic formula.



Chemical name. Leurocristine sulfate (1 : 1) (salt); 22-oxovincaleukoblastine sulfate (1 : 1) (salt); CAS Reg. No. 2068-78-2.

Description. A white to slightly yellow, amorphous or crystalline powder; odourless.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Vincristine sulfate should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Additional information. Vincristine sulfate is hygroscopic and very toxic. CAUTION: Vincristine sulfate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Vincristine sulfate contains not less than 95.0% and not more than 105.0% of $C_{46}H_{56}N_4O_{10},H_2SO_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A and D or tests B, C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum of the substance dried under reduced pressure for 16 hours at 40 °C is concordant with the spectrum obtained from vincristine sulfate RS similarly prepared or with the *reference spectrum* of vincristine sulfate.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution C.

C. To about 1 mg add 0.2 ml of vanillin/hydrochloric acid TS and allow to stand for approximately 1 minute; an orange colour is produced (distinction from vinblastine sulfate).

D. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Loss on drying. Dry at 40 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 16 hours; it loses not more than 120 mg/g.

pH value. pH of 1.0 g/ml solution, 3.5–4.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 40 volumes of toluene R, 20 volumes of chloroform R, and 3 volumes of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 0.20 mg of the test substance per ml, and (C) 10 mg of vincristine sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

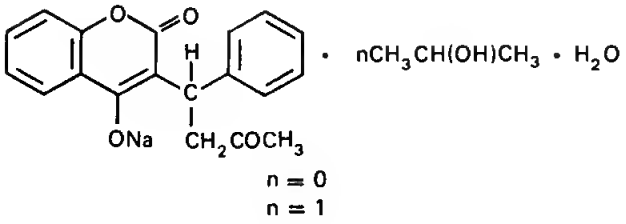
Assay. Dissolve about 10 mg, accurately weighed, in sufficient methanol R to produce 500 ml. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 297 nm and calculate the content of $C_{46}H_{56}N_4O_{10},H_2SO_4$, using the absorptivity value of 17.7 ($A^{1\%}_{1\,cm}$ = 177).

WARFARINUM NATRICUM
WARFARIN SODIUM

Molecular formula. $C_{19}H_{15}NaO_4$; $C_{19}H_{15}NaO_4,C_3H_8O,H_2O$ (hyclate).

Relative molecular mass. 330.3; 408.4 (hyclate).

Graphic formula.



Chemical name. 3-(α-Acetonylbenzyl)-4-hydroxycoumarin sodium salt; 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2*H*-1-benzopyran-2-one sodium salt; CAS Reg. No. 129-06-6.

3-(α-Acetonylbenzyl)-4-hydroxycoumarin sodium salt compound with 2-propanol monohydrate; 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2*H*-benzopyran-2-one sodium salt 2-propanol monohydrate.

Description. A white, amorphous or crystalline powder; odourless.

Solubility. Soluble in less than 1 part of water and in ethanol (~750 g/l) TS; slightly soluble in ether R.

Category. Anticoagulant.

Storage. Warfarin sodium should be kept in a well-closed container, protected from light.

Labelling. The designation on the container of Warfarin sodium should state whether the substance is in the amorphous or the crystalline, clathrate form.

Additional information. Warfarin sodium is discoloured by light. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Requirements

Definition. Warfarin sodium contains not less than 98.0% and not more than 102.0% of $C_{19}H_{15}NaO_4$, calculated with reference to the anhydrous and 2-propanol-free substance.

Identity tests

A. Dissolve 0.1 g in 25 ml of water, add 0.1 ml of hydrochloric acid (~70 g/l) TS, collect the precipitate on a filter (keep the filtrate for test C), wash with water, and dry the residue at 105 °C. Melting temperature, about 162 °C (warfarin). (Keep the residue for test B.)

B. Carry out the examination of the residue obtained in test A as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from warfarin RS or with the *reference spectrum* of warfarin.

C. The filtrate obtained in test A yields reaction B described under 2.1 General identification tests as characteristic of sodium.

D. Dissolve 1 g in 10 ml of water, add 5 ml of nitric acid (~1000 g/l) TS, and filter. To the filtrate add 2 ml of potassium dichromate (0.0167 mol/l) VS and shake for 5 minutes; only the clathrate yields a light greenish blue solution.

Clarity of solution. The opalescence of a solution of 0.50 g in 10 ml of carbon-dioxide-free water R is not more intense than that of opalescence standard TS2.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.4 g of the substance; the water content is not more than 45 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 7.2–8.3.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 5 volumes of chloroform R, 5 volumes of cyclohexane R, and 2 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 20 µl of each of 2 solutions in acetone R containing (A) 20 mg of the test substance per ml and (B) 0.020 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and immediately examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Absorbance in alkaline solution. Dissolve 1.25 g, accurately weighed, in 10 ml of sodium hydroxide (50 g/l) TS, filter through a membrane filter, and,

within 15 minutes, measure the absorbance of a 1-cm layer at the maximum at about 385 nm against a solvent cell containing sodium hydroxide (50 g/l) TS; not more than 0.3.

2-Propanol content. Dissolve about 0.8 g, accurately weighed, in 25.0 ml of water. Add 25.0 ml of sulfuric acid (0.125 mol/l) VS while swirling, filter, and transfer 10 ml of the clear filtrate to a 250-ml flask containing 40 ml of water. Add some boiling chips, then 30 ml of potassium dichromate TS3, and connect the flask to a condenser by means of a 75° connecting tube. Distil 60 ml, collecting the distillate in 20 ml of sodium hydroxide (~80 g/l) TS contained in a 250-ml iodine flask immersed in an ice-bath. Add, with swirling, 20.0 ml of iodine (0.1 mol/l) VS, insert the stopper in the flask, and allow to stand for 30 minutes. Add 5 ml of hydrochloric acid (~420 g/l) TS through the well in the flask, rinse the well and the neck of the flask with water, swirl to mix, remove the stopper, and titrate the excess iodine with sodium thiosulfate (0.1 mol/l) VS, adding 3 ml of starch TS towards the end of the titration. Each ml of iodine (0.1 mol/l) VS is equivalent to 1.001 mg of 2-propanol. The amorphous substance contains not more than 3 mg per g and the crystalline clathrate contains between 43 and 83 mg of 2-propanol per g.

Assay. Dissolve about 0.1 g, accurately weighed, in sufficient sodium hydroxide (0.01 mol/l) VS to produce 100 ml and dilute 10 ml to 1000 ml with sodium hydroxide (0.01 mol/l) VS. Measure the absorbance of a 1-cm layer of the diluted solution at the maximum at about 308 nm. Calculate the amount of $C_{19}H_{15}NaO_4$ in the substance being tested by comparison with warfarin RS, similarly and concurrently examined, taking into account that each mg of warfarin RS is equivalent to 1.071 mg of $C_{19}H_{15}NaO_4$. In an adequately calibrated spectrophotometer the absorbance of the reference solution should be 0.47 ± 0.03.

ZINCI OXYDUM
ZINC OXIDE

Molecular formula. ZnO

Relative molecular mass. 81.38

Chemical name. Zinc oxide; CAS Reg. No. 1314-13-2.

Description. A white or faintly yellowish white, very fine, amorphous powder, free from grittiness; odourless.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in hydrochloric acid (~70 g/l) TS.

Category. Mild astringent used topically as a protective.

Storage. Zinc oxide should be kept in a well-closed container.

Additional information. Zinc oxide gradually absorbs carbon dioxide from the air.

Requirements

Definition. Zinc oxide contains not less than 99.0% and not more than 100.5% of ZnO, calculated with reference to the freshly ignited substance.

Identity tests

A. Heat strongly a small amount of the substance; it assumes a yellow colour, which disappears on cooling.

B. Dissolve 20 mg in 2.0 ml of hydrochloric acid (~70 g/l) TS, add 0.15 ml of potassium ferrocyanide (45 g/l) TS; a greenish white precipitate is formed.

Arsenic. Use a solution of 1.6 g in 35 ml of hydrochloric acid (~70 g/l) TS and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 6 µg/g.

Carbonates and acid-insoluble substances. Mix 2.0 g with 10 ml of water, add 30 ml of sulfuric acid (~100 g/l) TS, and heat on a water-bath with constant stirring; no effervescence occurs and the resulting solution is clear and colourless.

Iron. Dissolve 0.20 g in 5 ml of hydrochloric acid (~250 g/l) TS and 30 ml of water. Treat the solution as described under 2.2.4 Limit test for iron; not more than 200 µg/g.

Lead. Add 2 g to 20 ml of water, stir well, add 5 ml of glacial acetic acid R, and warm on a water-bath until solution is effected. Then add 0.25 ml of potassium chromate (100 g/l) TS; no turbidity or precipitate is produced.

Loss on ignition. Ignite 1.0 g at 500 °C to constant weight; it loses not more than 10 mg/g.

Alkalinity. Mix 1 g with 10 ml of hot water, add 0.1 ml of phenolphthalein/ethanol TS and filter; if the filtrate is red, not more than 0.3 ml of hydrochloric acid (0.1 mol/l) VS is required to discharge the colour.

Assay. Dissolve about 0.15 g, accurately weighed, in 10 ml of acetic acid (~120 g/l) TS and proceed with the titration as described under 2.5 Complexometric titrations for zinc. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 4.069 mg of ZnO.

Monographs

Dosage Forms: General monographs

Dosage Forms: General monographs

CAPSULES

Definition

Capsules are solid dosage forms with hard or soft shells. They are of various shapes and sizes, and contain a single dose of one or more active ingredients. They are intended for oral administration, but preparations for alternative applications, such as vaginal or rectal, are also available in this presentation. These preparations may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph. Starch capsules (often known as cachets) are not described in this monograph.

The different categories of capsules that exist include hard, soft, and modified-release capsules. Their surfaces may bear symbols or other markings. They should be sufficiently robust to withstand handling, including packaging, storage, and transportation, without cracking or breaking. They should be packaged and stored in a manner that protects them from microbial contamination.

Capsule shells are made of gelatin or other substances, the consistency of which may be modified by the addition of substances such as glycerol and sorbitol. Preservatives may also be necessary. The shell should disintegrate in the presence of digestive fluids so that the contents are released. The contents of capsules may be solid, liquid, or of a paste-like consistency. Capsule shells and contents may contain excipients such as diluents, solvents, surface-active substances, opaque fillers, antimicrobial agents, sweeteners, colouring matter, flavouring substances, disintegrating agents, glidants, lubricants, and substances capable of modifying the behaviour of the active ingredient(s) in the gastrointestinal tract. The contents should not cause deterioration of the shell.

When excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between any of the components of the dosage form.

Visual inspection

Unpack and inspect at least 20 capsules. They should be smooth and undamaged. Evidence of physical instability is demonstrated by gross changes in physical appearance, including hardening or softening, cracking, swelling, mottling, or discoloration of the shell.

Uniformity of mass

Capsules comply with the test for 5.2 Uniformity of mass for single-dose preparations, unless otherwise specified in the individual monograph.

Uniformity of content

A requirement for compliance with the test for 5.1 Uniformity of content for single-dose preparations is specified in certain individual monographs where the active ingredient is 5% or less of the total formulation. In such cases the test for 5.2 Uniformity of mass for single-dose preparations is not required.

Dissolution test

Where a requirement for the "Dissolution test" is specified in the individual monograph, compliance with 5.3 Disintegration test for tablets and capsules is not required.

Labelling

Every pharmaceutical preparation must comply with the labelling requirements established under Good Manufacturing Practice.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); INNs should be used wherever possible;
(3) the amount of the active ingredient(s) in each capsule and the number of capsules in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;
(7) directions for use, warnings, and precautions that may be necessary; and
(8) the name and address of the manufacturer or the person responsible for placing the product on the market.

Storage

Capsules should be kept in well-closed containers. They should be protected from light, excessive moisture, or dryness, and should not be subjected to temperatures above 30 °C. Additional special packaging, storage, and transportation recommendations are specified in the individual monograph.

Requirements for specific types of capsules

Hard capsules

Definition

Hard capsules have shells consisting of two prefabricated cylindrical sections that fit together. One end of each section is rounded and closed, and the other is open. The contents of hard capsules are usually in solid form (powder or granules); in certain cases the contents may be in the form of encapsulated powders or micropellets.

Manufacture

The manufacturing and filling processes for hard capsules should meet the requirements of Good Manufacturing Practice, especially with regard to cross-contamination. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

The particle size of the active ingredient(s) is of primary significance in determining the rate and extent of dissolution, the bioavailability, and the uniformity of a drug product, especially for substances of low solubility in aqueous media. In order to obtain a suitable formulation, it is usually necessary to mix the active ingredient(s) with a number of excipients. It is essential that such mixing is carried out in a manner that ensures homogeneity. Sometimes, the physical characteristics of the mixture allow it to be directly filled into the shell, but it may occasionally be necessary to granulate before filling. Normally the granulate needs to be mixed with lubricants and/or disintegrating agents. The use of excessive amounts of lubricants should be avoided since these may deleteriously affect the capsules.

A uniform mass of the capsule mixture is volumetrically fed into the narrower lower section of the shell body which is then closed by slipping the larger section or cap over it. The security of the closure may be ensured by suitable means.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production. In-process controls during hard capsule production should include the particle size of the active ingredient(s), the homogeneity and moisture content of the mixture and/or granulate (as well as of the shells), the size of granules, the flow of the final mixture, and the uniformity of mass, capsule size, integrity of the seals, and disintegration or dissolution rate (e.g. for modified-release capsules) of the finished dosage form.

Disintegration test

Hard capsules comply with 5.3 Disintegration test for tablets and capsules.

Use water as the immersion fluid unless hydrochloric acid (0.1 mol/l) VS is specified in the individual monograph. Operate the apparatus for 30 minutes and examine the state of the capsules.

If capsules float, use a disc as described under 5.4 Disintegration test for suppositories.

Soft capsules

Definition

Soft capsules have thicker shells than hard capsules, and preservatives are usually added. The shells are of one piece and various shapes. Partial migration of the contents into the shell may occur (and vice versa) depending on the nature of the materials used and the product in question.

Manufacture

The manufacturing processes for soft capsules should meet the requirements of Good Manufacturing Practice. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

The particle size of the active ingredient(s) is of primary significance in determining the rate and extent of dissolution, the bioavailability, and the uniformity of a drug product, especially for substances of low solubility in aqueous media. In order to obtain a suitable formulation, it is usually necessary to mix the active ingredient(s) with a number of excipients. It is essential that such mixing is carried out in a manner that ensures homogeneity. Soft gelatin capsules are usually formed, filled, and sealed in one operation. However, shells for extemporaneous use are sometimes prefabricated. Liquids may be incorporated directly. Solids are usually dissolved or dispersed in a suitable excipient(s) to give a solution or dispersion of thick consistency.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production. In-process controls during soft capsule production should include the particle size of the active ingredient(s), the homogeneity of the mixture, the viscosity of the contents, and the uniformity of mass, capsule size, integrity of the seals, and disintegration or dissolution rate (e.g. for modified-release capsules) of the finished dosage form.

Disintegration test

Soft capsules comply with 5.3 Disintegration test for tablets and capsules, using water as the immersion fluid unless hydrochloric acid (0.1 mol/l) VS is specified in the individual monograph. Operate the apparatus for 30 minutes and examine the state of the capsules.

Modified-release capsules

Definition

Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain additives or are prepared by special procedures such as micro-encapsulation which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastrointestinal tract.

Extended-release capsules

Definition

Extended-release capsules are designed to slow the rate of release of the active ingredient(s) in the gastrointestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

Delayed-release capsules (enteric capsules)

Definition

Delayed-release capsules are hard or soft capsules prepared in such a manner that either the shell or the contents resist the action of gastric fluid but release the active ingredient(s) in the presence of intestinal fluid.

Manufacture

The statements given under either hard or soft capsules apply, as appropriate to delayed-release capsules.

Disintegration test

Delayed-release capsules comply with 5.3 Disintegration test for tablets and capsules, using hydrochloric acid (0.1 mol/l) VS as the immersion fluid. Operate the apparatus for 2 hours, unless otherwise specified in the individual monograph (but in any case for not less than 1 hour), and examine the state of the capsules. No capsule should show signs of disintegration or rupture permitting the contents to escape. Replace the acid by phosphate buffer solution, pH 6.8, TS with added pancreatin R where specified in the individual monograph. Operate the apparatus for 60 minutes and examine the state of the capsules.

OPHTHALMIC PREPARATIONS

Definition

Ophthalmic preparations (eye preparations) are sterile, liquid, semi-solid, or solid preparations that may contain one or more active pharmaceutical ingredient(s) intended for application to the conjunctiva, the conjunctival sac or the eyelids.

The choice of base and any excipients used for the preparation of ophthalmic preparations must be proven through product development studies not to affect adversely either the stability of the final product or the availability of the active ingredients at the site of action. The addition of colouring agents is not recommended.

Unless the active ingredient itself has antimicrobial activity, ophthalmic preparations supplied as multidose preparations may include a suitable antimicrobial agent. The antimicrobial activity should remain effective throughout the entire period of use.

The different categories of ophthalmic preparations include drops consisting of emulsions, solutions or suspensions, and ointments.

Manufacture

The manufacturing processes should meet the requirements of Good Manufacturing Practices, especially with regard to cross-contamination. The follow-

ing information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production. In-process controls during production of ophthalmic preparations should include monitoring environmental conditions (especially with respect to particulate and microbial contamination), pyrogens (use of a limulus amoebocyte lysate (LAL) test may be advantageous), pH and clarity of solution, and integrity of container (absence of leakage, etc.). Appropriate limits should be set for the particle size of the active ingredient(s).

It is essential that ophthalmic preparations are sterile. An aseptic manufacturing process is usually employed when the dosage form does not allow routine sterilization methods to be used.[1]

Packaging must be adequate to protect ophthalmic preparations from light, moisture, microbial contamination, and damage due to handling and transportation.

Visual inspection

Inspect the ointments, aqueous or oily solution, suspensions, or emulsions.

Evidence of physical and/or chemical instability is demonstrated by noticeable changes in colour and odour.

Sterility

Ophthalmic preparations comply with 3.2 Test for sterility.

Particle size

Ophthalmic preparations containing dispersed solid particles comply with the following test.

Take a quantity of the preparation (shake the container gently if necessary) corresponding to at least 10 µg of solid active ingredient and place in a counting cell or spread in a thin layer on a slide. Firmly apply a cover-glass and scan the whole area of the sample under a microscope.[2]

For each 10 µg of solid active substance not more than 20 particles should have a maximum dimension greater than 25 µm and not more than two of these particles should have a maximum dimension greater than 50 µm. None of the particles should have a maximum dimension greater than 90 µm.

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[1] 5.8 Methods of sterilization.

[2] For practical reasons, the whole sample is first scanned at low magnification (e.g. ×50) and particles >25 µm are identified. The larger particles can then be measured at a higher magnification (e.g. ×200–×500).

Containers

The materials for containers and closures should not adversely affect the quality of the preparation or allow diffusion of any kind into or across the material of the container into the preparation. The container should be fitted with a closure that minimizes microbial contamination and a device that reveals whether the container has ever been opened.

Labelling

Every pharmaceutical preparation must comply with the labelling requirements established by Good Manufacturing Practices.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); International Nonproprietary Names (INN) should be used wherever possible;
(3) the concentration(s) of the active ingredient(s) and the amount or the volume of preparation in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date, the utilization period, and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;
(7) if applicable, the period of use after opening the container;
(8) directions for use, warnings and precautions that may be necessary;
(9) the name and address of the manufacturer or the person responsible for placing the product on the market;
(10) if applicable, the name(s) and concentration(s) of antimicrobial agent(s) and/or antioxidant(s) incorporated in the preparation; and
(11) the statement "This preparation is sterile".

Storage

Ophthalmic preparations should maintain their integrity throughout their shelf-life when stored at the temperature indicated on the label. Special storage recommendations or limitations are indicated in individual monographs.

Requirements for specific types of ophthalmic preparations

Ophthalmic drops

Definition

Ophthalmic drops (eye drops) are sterile aqueous or oily solutions, suspensions, or emulsions intended for instillation into the conjunctival sac.

Ophthalmic drops should be clear and practically free from particles when examined under suitable conditions of visibility.

"Water for injections" should be used in the manufacture of aqueous ophthalmic drops.

The preparation of aqueous ophthalmic drops requires careful consideration of the need for isotonicity, a certain buffering capacity, the desired pH, the addition of antimicrobial agents and/or antioxidants, the use of viscosity-increasing agents, and the choice of appropriate packaging.

Ophthalmic drops are considered isotonic when the tonicity is equal to that of a 0.9% solution of sodium chloride. The eye can usually tolerate solutions equivalent to 0.5–1.8% of sodium chloride.

Ideally, the pH of ophthalmic drops should be equivalent to that of tear fluid, which is 7.4. However, the decision to add a buffering agent should be based on stability considerations. The pH selected should be the optimum for both stability of the active pharmaceutical ingredient and physiological tolerance. If a buffer system is used, it must not cause precipitation or deterioration of the active ingredient. The influence on the lachrymal flow should also be taken into account.

Visual inspection

Evidence of physical instability is demonstrated by the cloudiness of aqueous solutions, due to the formation of a precipitate.

Containers

Ophthalmic drops are normally supplied in suitable multidose containers that allow successive drops of the preparation to be administered. The container should be fitted with a tamper-evident device. The maximum volume of the preparation in such a container should be no more than 10 ml, unless otherwise specified and authorized. Multidose ophthalmic drop preparations may be used for up to 4 weeks after the container is initially opened. Droppers supplied separately should also comply with 3.2 Test for sterility.

Ophthalmic drops may also be provided in suitable single-dose containers that will maintain the sterility of the contents and the applicator up to the time of use.

It is recommended that single-dose containers for surgical use should not include any antimicrobial agents.

Ophthalmic emulsions

Definition

Ophthalmic emulsions are generally dispersions of oily droplets in an aqueous phase. There should be no evidence of breaking or coalescence.

Ophthalmic suspensions

Definition

Ophthalmic suspensions contain solid particles dispersed in a liquid vehicle; they must be homogeneous when shaken gently and remain sufficiently dispersed to enable the correct dose to be removed from the container. A sediment may occur, but this should disperse readily when the container is shaken, and the size of the dispersed particles should be controlled. The active ingredient and any other suspended material must be reduced to a particle size small enough to prevent irritation and damage to the cornea.

Visual inspection

Evidence of physical instability is demonstrated by the formation of agglomerates or precipitates in aqueous solutions (suspensions) that do not disperse when the solution is shaken gently.

Ophthalmic ointments

Definition

Ophthalmic ointments are sterile, homogeneous, semi-solid preparations intended for application to the conjunctiva or the eyelids.

They are usually prepared from non-aqueous bases, e.g. soft paraffin (Vaseline), liquid paraffin, and wool fat. They may contain suitable additives, such as antimicrobial agents, antioxidants, and stabilizing agents.

Organoleptic inspection

Evidence of physical instability is demonstrated by:

— a noticeable change in consistency, such as excessive "bleeding" (separation of excessive amounts of liquid) or formation of agglomerates or grittiness;
— discoloration;
— emulsion breakdown;
— crystal growth;
— shrinking due to evaporation of water; or
— evidence of microbial growth.

Uniform consistency

Ophthalmic ointments should be of uniform consistency. When a sample is rubbed on the back of the hand, no solid components should be noticed.

Containers

Ophthalmic ointments are normally supplied in small, sterilized, collapsible tubes fitted with a tamper-evident applicator. The containers or the nozzles

of the tubes are shaped so that the ointment can be applied without contaminating what remains in the tube. The content of such a container is limited to not more than 5 g of the preparation.

Suitable single-dose containers may also be used.

PARENTERAL PREPARATIONS

Definition

Parenteral preparations are sterile, pyrogen-free liquids (solutions, emulsions, or suspensions) or solid dosage forms containing one or more active ingredients, packaged in either single-dose or multidose containers. They are intended for administration by injection, infusion, or implantation into the body.

Preparations such as vaccines, human blood and products derived from human blood, peritoneal dialysis solutions, and radioactive pharmaceuticals require special formulation, methods of manufacture, or presentation appropriate to their particular use and may not comply with certain parts of this monograph.

There are four main forms of parenteral preparations: injections, intravenous infusions (large volume parenterals), powders for injections, and implants. Certain injections and intravenous infusions may be presented in the form of sterile concentrated solutions, which must be suitably diluted before use.

Parenteral preparations may contain excipients such as solvents, suspending agents, buffering agents, substances to make the preparation isotonic with blood, stabilizers, or antimicrobial preservatives. The addition of excipients should be kept to a minimum. When excipients are used, they should not adversely affect the stability, bioavailability, safety, or efficacy of the active ingredient(s), or cause toxicity or undue local irritation. There must be no incompatibility between any of the components of the dosage form.

Water for injections is used as the vehicle for aqueous injections. It should be freshly distilled by the process described under "Aqua pro Injectione", be free from carbon dioxide, and comply with 3.4 Test for bacterial endotoxins. Sterilization at this stage may be omitted, provided that the solution or preparation is immediately sterilized upon finalization. For non-aqueous injections, fixed oils of vegetable origin are used as vehicles.

Unless otherwise specified in the individual monograph, sodium chloride or other suitable substance(s), may be added to an aqueous solution for injection in order to render the preparation isotonic.

Manufacture

The manufacturing process should meet the requirements of Good Manufacturing Practice. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

The quality of starting materials, the design and maintenance of the equipment, and the method of manufacture must be such as to ensure the stability of the active substance and the final product which is sterile and free of pyrogens and particulate matter. From the clinical viewpoint all parenteral preparations must be pyrogen-free. For practical purposes, however, certain categories of parenteral preparations may be exempted from the test for bacterial endotoxins or the test for pyrogens as specified in the individual monograph.

For the sterilization of parenteral preparations follow 5.8 Methods of sterilization. Heating in an autoclave is the method of choice for aqueous preparations and should therefore be used whenever possible.

When a parenteral preparation is liable to deterioration due to oxidation, the operation of filling may be performed in an atmosphere of suitable inert gas, such as nitrogen, whereby the air in the container is replaced by this gas.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production. In-process controls during production of parenteral preparations should include monitoring of environmental conditions (especially with respect to particulate and microbial contamination), bacterial endotoxins, pH and clarity of solution, freedom from particulate matter, and integrity of container (absence of leakage, etc.). For dispersions controls should also include the particle size of the dispersed phase, and for powders for injections the uniformity of content and mass, moisture content, and the ease of reconstitution of a solution or suspension. The presence of preservatives or other additives should be determined as these can influence the choice of assay method.

Visual inspection

Inspect the solutions, reconstituted solutions, and intravenous infusions (except dispersions). They should be clear and free from visible particulate matter.

Test for sterility

Parenteral preparations comply with 3.2 Test for sterility.

Test for bacterial endotoxins/pyrogens

All intravenous infusions and those injections and powders for injections where the volume to be injected in a single dose is 15 ml or more must comply with 3.4 Test for bacterial endotoxins or, where justified, with 3.5 Test for pyrogens. In addition, where specified in the individual monographs for certain preparations where the active ingredients are of biological origin, injections and powders for injections must comply with the appropriate test, irrespective of the volume to be injected in a single dose.

For injections, the amount to be tested should relate to the volume of the dose and should be specified in the individual monograph.

For powders for injections, the amount of powder to be tested and the nature and volume of the liquid in which it is to be dissolved or suspended should be specified in the individual monograph.

Containers

Parenteral preparations are usually supplied in glass ampoules, bottles or vials, plastic bottles or bags, and prefilled syringes, which are coloured in the case of light-sensitive substances.

Except where otherwise indicated in individual monographs, these containers should be made from material that is sufficiently transparent to permit the visual inspection of the contents. They should not adversely affect the quality of the preparation, allow diffusion of any kind into or across the material of the container, or yield foreign substances into the preparation.

Closures

Closures for parenteral preparation containers should be equipped with a firm seal to prevent entry of microorganisms and other contaminants while permitting the withdrawal of a part or the whole of the contents without removal of the closure. They should not be made of components that react with the contents, nor should they allow foreign substances to diffuse into the preparation. Plastic materials or elastomers of which the closure is composed should be sufficiently firm and elastic to allow the passage of a needle with the least possible shedding of particles. Closures for multidose containers should be sufficiently elastic to allow the puncture to reseal when the needle is withdrawn and protect the contents from airborne contamination. A tamper-evident container is fitted with a device that reveals clearly whether it has ever been opened.

Labelling

Every pharmaceutical preparation must comply with the labelling requirements established under Good Manufacturing Practice.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); INNs should be used wherever possible;
(3) the amount of the active ingredient(s) in a suitable dose volume and the volume in the container; for powder for injections: the amount of the active ingredient(s) in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;

(7) directions for use, warnings, and precautions that may be necessary; and
(8) the name and address of the manufacturer or the person responsible for placing the product on the market.

For parenteral preparations that are solutions or dispersions, the concentration of the active ingredient(s) should be given in terms of mass or biological activity per volume. For concentrated solutions, labels should state the composition and the dilution to be carried out before use.

Requirements for specific types of parenteral preparations

Injections

Definition

Injections are sterile, pyrogen-free solutions or dispersions (emulsions or suspensions) of one or more active ingredients in a suitable vehicle.

Whenever possible, an injection should be prepared using an aqueous vehicle. If necessary, suitable non-aqueous solvents are indicated in the individual monographs. Injections that are dispersions should remain sufficiently stable so that, after shaking, a homogeneous dose can be withdrawn.

The use of single-dose injections is to be preferred and is essential when the preparation is intended for administration by routes where, for medical reasons, an antimicrobial preservative is not acceptable, e.g. intracisternal, intrathecal.

Single-dose preparations

Single-dose preparations should contain a sufficient quantity of the injection readily to permit the withdrawal and administration of the volume specified on the label.

Multidose preparations

Multidose preparations should contain a suitable antimicrobial preservative in appropriate concentrations, except in cases where the preparations themselves have adequate antimicrobial properties. The containers should be equipped to ensure adequate protection of the contents after partial withdrawal. In order to minimize the risk of contamination resulting from multiple penetrations of the closure, the contents of a multidose preparation should normally not exceed 30 ml.

Intravenous infusions

Definition

Intravenous infusions are sterile, pyrogen-free aqueous solutions or emulsions with water as continuous phase, usually prepared to be isotonic. They are intended for administration in large volumes (usually 100 ml or more), and should not contain any antimicrobial preservatives.

On visual inspection, emulsions for intravenous injection should show no evidence of phase separation. The particle size of the dispersed phase should be controlled by the manufacturer.

Powders for injections

Definition
Powders for injections are solid substances (including freeze-dried materials), distributed in their final containers and which, when shaken with the prescribed volume of the appropriate sterile liquid, rapidly form either clear and practically particle-free solutions or uniform suspensions. Powders for injections, after dissolution or suspension, comply with the requirements for injections or intravenous infusions, as appropriate.

Uniformity of mass
Powders for injections (single-dose use) comply with the test for 5.2 Uniformity of mass for single-dose preparations, unless otherwise specified in the individual monograph.

Uniformity of content
A requirement for compliance with the test for 5.1 Uniformity of content for single-dose preparations is specified in certain individual monographs where the active ingredient is less than 40 mg. In such cases, compliance with the test for 5.2 Uniformity of mass for single-dose preparations may not be required.

Implants

Definition
Implants are solid preparations containing one or more active ingredients. They are of a size and shape suitable for parenteral implantation, and provide release of the active ingredient(s) over an extended period of time. They are presented in individual sterile containers.

All requirements for these specialized dosage forms are given in the individual monographs.

SUPPOSITORIES

Definition
Suppositories are solid preparations which may contain one or more active pharmaceutical ingredient(s) intended for rectal application. They are normally used for local action or systemic absorption of the active ingredient(s). They usually melt, soften, or dissolve at body temperature.

Suppositories are usually prepared from excipients or bases such as cocoa butter, hard fat, glycerinated gelatin, hydrogenated vegetable oils and macrogols. They may also contain additives, such as adsorbents, surface-active agents, viscosity-influencing agents, antioxidants, antimicrobials, and authorized colouring agents.

Any excipients used for the preparation of suppositories must be proven through product development studies not to affect adversely either the stability of the final product or the availability of the active ingredient(s) at the site of action; incompatibility between any of the components of the dosage form should be avoided.

Manufacture

The manufacturing processes should meet the requirements of Good Manufacturing Practices, especially with regard to cross-contamination. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production.

Where the active ingredient is suspended in the suppository base, appropriate limits should be set for the particle size.

Suppositories may be manufactured by moulding or compressing powdered material into a suitable shape, or by encapsulating a semi-solid mass into soft gelatin. Rectal capsules are in general similar to soft capsules, except that they may have lubricating coatings.

Moulded suppositories are the most common type. They are usually obtained by pouring the medicated mass, sufficiently liquefied by heating, into suitable moulds. The suppositories solidify on cooling. In certain cases, it is also possible to use the cold-moulding compression procedure in a suitable press.

Packaging must be adequate to protect suppositories from light, excessive temperature, moisture, and damage due to handling and transportation. It is necessary to ensure that the suppositories can be released from the packaging material easily and without damage.

Visual inspection

Suppositories are elongated, smooth and have a uniform texture and appearance. They may also consist of several layers.

Evidence of physical and/or chemical instability is demonstrated by noticeable changes in:

— surface texture or form; and
— colour and odour.

Disintegration
Suppositories should comply with 5.4 Disintegration test for suppositories, unless intended for modified release. Unless otherwise stated in the individual monograph, for each of the three suppositories, examine the state of the sample after 30 minutes for fat-based suppositories and rectal capsules, and after 60 minutes for water-soluble based suppositories.

Uniformity of mass
See the general requirements under 5.2 Uniformity of mass for single-dose preparations. Not more than two of the individual masses should deviate from the average mass by more than 5%, and none by more than 10%.

Uniformity of content
See the general requirements under 5.1 Uniformity of content for single-dose preparations. Preparations with an active ingredient content of less than 2 mg, or less than 2% of the total mass, comply with the test, unless otherwise described in the individual monograph and authorized. If the preparation has more than one active ingredient, the requirement applies only to those active ingredients that fall into the above category. If the test for uniformity of content is prescribed for all active ingredients, the test for uniformity of mass is not required.

Containers
Suppositories should be supplied in a well-closed container. The container material should not adversely affect the quality of the preparation, nor should it allow diffusion into or across the material of the container or yield foreign substances into the preparation.

Labelling
Every pharmaceutical preparation must comply with the labelling requirements established by Good Manufacturing Practices.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); International Nonproprietary Names (INN) should be used wherever possible;
(3) the amount of the active ingredient(s) in each suppository and the number of suppositories in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;
(7) directions for use, warnings and precautions that may be necessary;

(8) the name and address of the manufacturer or the person responsible for placing the product on the market; and
(9) if applicable, the names and concentrations of the antimicrobial agents and/or antioxidants incorporated in the preparation.

Storage
Suppositories should maintain their shape throughout their shelf-life when stored at the temperature indicated on the label.

TABLETS

Definition
Tablets are solid dosage forms containing one or more active ingredients. They are obtained by single or multiple compression (in certain cases they are moulded) and may be uncoated or coated. They are usually intended for oral administration, but preparations for alternative applications, such as implants, solution-tablets for injections, irrigations, or for external use, vaginal tablets, etc., are also available in this presentation. These preparations may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph.

The different categories of tablet that exist include soluble tablets, effervescent tablets, tablets for use in the mouth, and modified-release tablets. Unless otherwise specified in the individual monograph, tablets are normally circular in shape, and their surfaces are flat or convex. Tablets may have lines or break-marks, symbols, or other markings. They should be sufficiently hard to withstand handling, including packaging, storage, and transportation, without crumbling or breaking.

Tablets may contain excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the dosage forms and the active ingredient(s) in the gastrointestinal tract, colouring matter, and flavouring substances. When such excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between any of the components of the dosage form.

Manufacture
The manufacturing processes should meet the requirements of Good Manufacturing Practice, especially with regard to cross-contamination. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

The particle size of the active ingredient(s) is of primary significance in determining the rate and extent of dissolution, the bioavailability, and the uniformity of a drug product, especially for substances of low solubility in aqueous media. In order to obtain a suitable formulation, it is usually necessary to mix the active ingredient(s) with a number of excipients. It is essential that such mixing is carried out in a manner that ensures homogeneity. Sometimes, the physical characteristics of the mixture allow it to be directly compressed, but it is usually necessary to granulate before compression, either by wet granulation or precompression (slugging).

The granulate and powders normally need to be mixed with lubricants and/or disintegrating agents. The use of excessive amounts of lubricants should be avoided since these will deleteriously affect the tablets. The final tablet mixture is volumetrically fed into the die cavity to ensure that the tablets are of a uniform mass when compressed. The tablets may be coated, either in coating pans or by an air-suspension technique. A hydrophobic subcoat applied to the core of sugar-coated tablets may reduce dissolution.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production. In-process controls during tablet production should include the particle size of the active ingredient(s), the homogeneity and moisture content of the mixture and/or granulate, the size of granules, the flow of the final mixture, and the dimensions (thickness, diameter), uniformity of mass, hardness and/or crushing force, friability, disintegration, or dissolution rate (e.g. for modified-release tablets) of the finished dosage form. Attention should be paid to the uniformity of mass of tablet cores before coating.

Packaging must be adequate to protect the tablets from light, moisture, and damage during packaging and transportation.

Visual inspection

Unpack and inspect at least 20 tablets. They should be undamaged, smooth, and usually of uniform colour.

Evidence of physical instability is demonstrated by:

— presence of excessive powder and/or pieces of tablets at the bottom of the container (from abraded, crushed, or broken tablets);
— cracks or capping, chipping in the tablet surfaces or coating, swelling, mottling, discoloration, fusion between tablets;
— the appearance of crystals on the container walls or on the tablets.

Uniformity of mass

Tablets comply with the test for 5.2 Uniformity of mass for single-dose preparations, unless otherwise specified below or in the individual monograph.

Uniformity of content
A requirement for compliance with the test for 5.1 Uniformity of content for single-dose preparations is specified in certain individual monographs for sugar-coated or enteric-coated tablets, where the test for 5.2 Uniformity of mass for single-dose preparations does not apply. In addition, a requirement is specified in certain individual monographs where the active ingredient is 5% or less of the total formulation. In such cases the test for 5.2 Uniformity of mass for single-dose preparations is not required.

Dissolution test
Where a requirement for the "Dissolution test" is specified in the individual monograph, the 5.3 Disintegration test for tablets and capsules is not required.

Labelling
Every pharmaceutical preparation must comply with the labelling requirements established under Good Manufacturing Practice.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); International Nonproprietary Names (INN) should be used wherever possible;
(3) the amount of the active ingredient(s) in each tablet and the number of tablets in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;
(7) directions for use, warnings, and precautions that may be necessary; and
(8) the name and address of the manufacturer or the person responsible for placing the product on the market.

Storage
Tablets should be kept in well-closed containers and protected from light, moisture, crushing, and mechanical shock. Any special storage conditions should be stated on the label. Tablets should be able to withstand handling, including packaging and transportation, without losing their integrity. Moisture-sensitive forms, such as effervescent tablets, should be stored in tightly closed containers or moisture-proof packs and may require the use of separate packages containing water-adsorbent agents, such as silica gel.

Additional special packaging, storage, and transportation recommendations are specified in the individual monograph.

Requirements for specific types of tablets

Uncoated tablets

Definition
The majority of uncoated tablets are made in such a way that the release of active ingredients is unmodified. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multilayer tablets), but no signs of coating.

Disintegration test
Uncoated tablets, except effervescent tablets, tablets for use in the mouth, and chewable tablets, comply with 5.3 Disintegration test for tablets and capsules. Operate the apparatus for 15 minutes, unless otherwise specified in the individual monograph, and examine the state of the tablets.

Soluble tablets (tablets for solutions)

Definition
Soluble tablets are uncoated tablets that dissolve in water to give a clear solution.

Disintegration test
Soluble tablets comply with 5.3 Disintegration test for tablets and capsules. Use water at room temperature, and operate the apparatus for 5 minutes, unless otherwise specified in the individual monograph.

Effervescent tablets

Definition
Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates that react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Labelling
The label should state: "Not to be swallowed directly".

Disintegration test
Effervescent tablets comply with 5.3 Disintegration test for tablets and capsules. Place one tablet in a 250-ml beaker containing 200 ml of water at room temperature. Numerous bubbles of gas are evolved. When the evolution of gas around the tablet or its fragments ceases, the tablet should have disintegrated, being either dissolved or dispersed in the water so that no agglomerates remain. Repeat the operation on five additional tablets. The tablets comply with the

test if each of the six tablets used in the test disintegrates within 5 minutes, unless otherwise specified in the individual monograph.

Tablets for use in the mouth (sublingual, buccal) and chewable tablets

Definition

Tablets for use in the mouth and chewable tablets are usually uncoated. They are formulated to effect a slow release and local action of the active ingredient(s) (for example, compressed lozenges) or the release and absorption of the active ingredient(s) under the tongue (sublingual tablets) or in other parts of the mouth (buccal) for systemic action.

Coated tablets

Definition

Coated tablets are tablets covered with one or more layers of mixtures of substances such as natural or synthetic resins, polymers, gums, fillers, sugars, plasticizers, polyols, waxes, colouring matters, flavouring substances, and sometimes also active ingredients. A broken section, when examined under a lens, shows a core which is surrounded by a continuous layer of a different texture.

The tablets may be coated for a variety of reasons such as protection of the active ingredients from air, moisture, or light, masking of unpleasant tastes and odours, or improvement of appearance. The substance used for coating is usually applied as a solution or suspension.

Three main categories of coated tablet may be distinguished: sugar-coated, film-coated, and certain modified-release tablets.

Sugar-coated tablets

Uniformity of mass

The test for 5.2 Uniformity of mass for single-dose preparations, does not apply to sugar-coated tablets (see in-process controls under "Manufacture").

Disintegration test

Sugar-coated tablets comply with 5.3 Disintegration test for tablets and capsules. Operate the apparatus for 60 minutes, unless otherwise specified in the individual monograph, using water, and examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on an additional six tablets, using hydrochloric acid (0.1 mol/l) VS.

All six tablets must disintegrate.

Film-coated tablets

Definition

A film-coated tablet is covered with a thin layer of resins, polymers, and/or plasticizers capable of forming a film.

Disintegration test
Film-coated tablets comply with 5.3 Disintegration test for tablets and capsules. Operate the apparatus for 30 minutes, and examine the state of the tablets.

Modified-release tablets

Definition
Modified-release tablets are coated, uncoated, or matrix tablets containing excipients or prepared by procedures which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastrointestinal tract.

Extended-release tablets

Definition
Extended-release tablets are designed to slow the rate of release of the active ingredient(s) in the gastrointestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

Delayed-release tablets (enteric-coated tablets)

Definition
Delayed-release tablets are intended to resist gastric fluid but disintegrate in intestinal fluid. This is achieved by using coating substances such as cellacefate (cellulose acetate phthalate) and anionic copolymers of methacrylic acid and its esters. It is sometimes necessary to apply more than one layer.

Uniformity of mass
The test for 5.2 Uniformity of mass for single-dose preparations does not apply to delayed-release tablets.

Disintegration test
Delayed-release tablets comply with 5.3 Disintegration test for tablets and capsules, using hydrochloric acid (0.1 mol/l) VS as the immersion fluid. Operate the apparatus for 2 hours, unless otherwise specified in the individual monograph (but in any case for not less than 1 hour), and examine the state of the tablets. No tablet should show signs of either disintegration (apart from fragments of coating) or cracks that would allow the contents to escape. Replace the acid by phosphate buffer solution, pH 6.8, TS. Operate the apparatus for 60 minutes and examine the state of the tablets.

TOPICAL SEMI-SOLID DOSAGE FORMS

Definition

Topical semi-solid dosage forms are normally presented in the form of creams, gels, ointments, or pastes. They contain one or more active ingredients dissolved or uniformly dispersed in a suitable base and any suitable excipients such as emulsifiers, viscosity-increasing agents, antimicrobial agents, antioxidants, or stabilizing agents. Preparations susceptible to the growth of microorganisms should contain a suitable antimicrobial agent in an appropriate concentration unless the preparations themselves have adequate antimicrobial properties. Assurance must be provided through product development studies that such excipients do not adversely affect either the stability of the final product or the availability of the active ingredient(s) at the site of action; there must be no incompatibility between any of the components of the dosage form.

The choice of a base for semi-solid dosage forms depends on many factors: the therapeutic effect desired, the nature of the active ingredient(s) to be incorporated, the availability of the active ingredient(s) at the site of action, the shelf-life of the finished product, and the environmental conditions in which the product is intended to be administered. In many cases, a compromise has to be made in order to achieve the required stability. For example, drugs that hydrolyse rapidly are more stable in hydrophobic bases than in water-containing bases, even though they may be more effective in the latter.

The base should neither irritate nor sensitize the skin, nor should it delay wound healing. It should be smooth, inert, odourless, physically and chemically stable, and compatible with both the skin and the active ingredient(s) to be incorporated. It should normally be of such a consistency that it spreads and softens easily when stress is applied.

It may be necessary for a topical semi-solid dosage form to be sterile, for example, when it is intended for use on large open wounds or severely injured skin.

Manufacture

The manufacturing processes should meet the requirements of Good Manufacturing Practice. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. They should be designed to guarantee the effectiveness of each stage of production. Appropriate limits should be set for the particle size of the active ingredient(s), which should be controlled during production. Particular care should be paid to environmental conditions, especially with respect to microbial and cross-contamination.[1]

[1] *Warning:* Semi-solid dosage forms should not be diluted. If a dilution is nevertheless necessary, this requires special attention; the same type of base should be used in order to obtain a homogeneous mixture.

Packaging must be adequate to protect topical semi-solid dosage forms from light, moisture, and damage due to handling and transportation. The use of flexible tubes of suitable metal or plastic is preferred. Preparations for nasal, aural, vaginal, or rectal use should be supplied in containers adapted for appropriate delivery of the product to the site of application, or should be supplied with a suitable applicator.

Organoleptic inspection
Evidence of physical instability is demonstrated by:

— a noticeable change in consistency, such as excessive "bleeding" (separation of excessive amounts of liquid) or formation of agglomerates and grittiness;
— discoloration;
— emulsion breakdown;
— crystal growth;
— shrinking due to evaporation of water; or
— evidence of microbial growth.

A noticeable change in odour is also a sign of instability.

Sterility
Preparations required to be sterile should comply with 3.2 Test for sterility.

Uniform consistency
Topical semi-solid dosage forms should be of uniform consistency. When a sample is rubbed on the back of the hand, no solid components should be noticed.

Containers
The container material should not adversely affect the quality of the preparation or allow diffusion of any kind into or across the material of the container into the preparation. The container should be fitted with a closure that minimizes microbial contamination and is equipped with a device that reveals whether the container has ever been opened.

Labelling
Every pharmaceutical preparation must comply with the labelling requirements established under Good Manufacturing Practice.

The label should include:

(1) the name of the pharmaceutical product;
(2) the name(s) of the active ingredient(s); INNs should be used wherever possible;

(3) the amount of the active ingredient(s) in a specified quantity of suitable base or vehicle, and the quantity of preparation in the container;
(4) the batch (lot) number assigned by the manufacturer;
(5) the expiry date and, when required, the date of manufacture;
(6) any special storage conditions or handling precautions that may be necessary;
(7) directions for use, warnings, and precautions that may be necessary;
(8) the name and address of the manufacturer or the person responsible for placing the product on the market;
(9) the name and quantity of antimicrobial agent incorporated in the preparation; and
(10) if applicable, the statement that the preparation is "sterile".

Storage
Topical semi-solid dosage forms should be kept in well-closed containers. The preparation should maintain its pharmaceutical integrity throughout shelf-life when stored at the temperature indicated on the label; the temperature should normally not exceed 25 °C. Special storage recommendations or limitations are indicated in individual monographs.

Requirements for specific types of topical semi-solid dosage forms

Creams

Definition
Creams are homogeneous, semi-solid preparations consisting of opaque emulsion systems. Their consistency and rheological properties depend on the type of emulsion, either water-in-oil (w/o) or oil-in-water (o/w), and on the nature of the solids in the internal phase. Creams are intended for application to the skin or certain mucous membranes for protective, therapeutic, or prophylactic purposes, especially where an occlusive effect is not necessary. The term "cream" is most frequently used to describe soft, cosmetically acceptable types of preparations.

Generally, o/w creams are prepared at an elevated temperature and then cooled down to room temperature in order for the internal phase to solidify. The semi-solid form of a w/o cream is attributable to the character of the external phase.

Hydrophobic creams (w/o)
Hydrophobic creams are usually anhydrous and absorb only small amounts of water. They contain w/o emulsifying agents such as wool fat, sorbitan esters, and monoglycerides.

Hydrophilic creams (o/w)

Hydrophilic creams contain bases that are miscible with water. They also contain o/w emulsifying agents such as sodium or triethanolamine soaps, sulfated fatty alcohols, and polysorbates combined, if necessary, with w/o emulsifying agents. These creams are essentially miscible with skin secretions.

Gels

Definition

Gels are usually homogeneous, clear, semi-solid preparations consisting of a liquid phase within a three-dimensional polymeric matrix with physical or sometimes chemical cross-linkage by means of suitable gelling agents.

Gels are applied to the skin or certain mucous membranes for protective, therapeutic, or prophylactic purposes.

Hydrophobic gels

Hydrophobic gel (oleogel) bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels

Hydrophilic gel (hydrogel) bases usually consist of water, glycerol, or propylene glycol gelled with suitable agents such as tragacanth, starch, cellulose derivatives, carboxyvinyl polymers, and magnesium aluminium silicates.

Ointments[1]

Definition

Ointments are homogeneous, semi-solid preparations intended for external application to the skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired.

Ointments are formulated using hydrophobic, hydrophilic, or water-emulsifying bases to provide preparations that are immiscible, miscible, or emulsifiable with skin secretions. They can also be derived from hydrocarbon (fatty), absorption, water-removable, or water-soluble bases.

Hydrophobic ointments

Hydrophobic (lipophilic) ointments are usually anhydrous and can absorb only small amounts of water. Typical bases used for their formulation are water-insoluble hydrocarbons such as hard, soft, and liquid paraffin, vegetable oil, animal fats, waxes, synthetic glycerides, and polyalkylsiloxanes.

[1] Ophthalmic ointments are described in the separate monograph for "Ophthalmic Preparations".

Water-emulsifying ointments

Water-emulsifying ointments can absorb large amounts of water. They typically consist of a hydrophobic fatty base in which a w/o agent, such as wool fat, wool alcohols, sorbitan esters, monoglycerides, or fatty alcohols can be incorporated to render them hydrophilic. They may also be w/o emulsions that allow additional quantities of aqueous solutions to be incorporated. Such ointments are used especially when formulating aqueous liquids or solutions.

Hydrophilic ointments

Hydrophilic ointment bases are miscible with water. The bases are usually mixtures of liquid and solid polyethylene glycols (macrogols).

Pastes

Definition

Pastes are homogeneous, semi-solid preparations containing high concentrations of insoluble powdered substances (usually not less than 20%) dispersed in a suitable base. The pastes are usually less greasy, more absorptive, and stiffer in consistency than ointments because of the large quantity of powdered ingredients present. Some pastes consist of a single phase, such as hydrated pectin, and others consist of a thick, rigid material that does not flow at body temperature. The pastes should adhere well to the skin. In many cases they form a protective film that controls the evaporation of water.

Monographs

Dosage Forms: Specific monographs

Dosage Forms: Specific monographs

ACIDI ACETYLSALICYLICI COMPRESSI
ACETYLSALICYLIC ACID TABLETS

Category. Analgesic; antipyretic; nonsteroidal antiinflammatory; antimigraine drug.

Storage. Acetylsalicylic acid tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 100–500 mg.

Usually no odour of acetic acid is perceptible on opening the container.

Requirements

Comply with the monograph for "Tablets".

Acetylsalicylic acid tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_9H_8O_4$ stated on the label.

Identity tests

A. Place a quantity of the powdered tablets equivalent to 10 mg of Acetylsalicylic acid on a suitable white test plate or on a watch-glass placed on a white background, and add 1 drop of ferric chloride (25 g/l) TS; no violet colour is produced.

B. Place a quantity of the powdered tablets equivalent to 10 mg of Acetylsalicylic acid on a suitable white test plate or a watch-glass placed on a white background, and add 1 drop of potassium hydroxide/ethanol TS1. After 1 minute add 1 drop of ferric chloride (25 g/l) TS; a violet colour is produced.

Salicylic acid. To a quantity of the powdered tablets equivalent to 0.2 g of Acetylsalicylic acid add 4 ml of ethanol (~750 g/l) TS and shake. Dilute to 100 ml with cool water (not exceeding 10 °C) and filter immediately. Transfer 50 ml of the filtrate to a comparison tube, add 1 ml of freshly prepared ferric ammonium sulfate TS1, mix, and allow to stand for 1 minute. Separately and concurrently, place 3 ml of a freshly prepared solution of salicylic acid R containing 0.1 mg per ml into a second comparison tube, add 2 ml of ethanol (~750 g/l) TS, 1 ml of freshly prepared ferric ammonium sulfate TS1, and sufficient water to produce 50 ml.

Any violet colour produced in the first tube is not more intense than that produced in the second tube (0.3%).

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.5 g of Acetylsalicylic acid, accurately weighed, add 30 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS, and boil gently for 10 minutes. Back-titrate the excess alkali with hydrochloric acid (0.5 mol/l) VS, using phenol red/ethanol TS as indicator. Repeat the procedure without the powdered tablets being examined and make any necessary corrections.

Each ml of carbonate-free sodium hydroxide (0.5 mol/l) VS is equivalent to 45.04 mg of $C_9H_8O_4$.

ALLOPURINOLI COMPRESSI
ALLOPURINOL TABLETS

Category. Drug used for the treatment of gout.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg.

Requirements

Comply with the monograph for "Tablets".

Allopurinol tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_5H_4N_4O$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Triturate a quantity of the powdered tablets equivalent to about 0.1 g of Allopurinol with 10 ml of sodium hydroxide (0.1 mol/l) VS. Filter, acidify the filtrate with acetic acid (~60 g/l) TS, and allow to stand for 10–15 minutes. Separate the precipitate, wash it with 3 ml of dehydrated ethanol R and 4 ml of ether R. Allow to dry in air for 15 minutes, then dry at 105 °C for 3 hours. Keep half of the residue for test C. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from allopurinol RS or with the *reference spectrum* of allopurinol.

B. The absorption spectrum of the solution obtained in the "Assay", when observed between 230 nm and 350 nm, exhibits a maximum at about 250 nm.

C. To the residue obtained in test A add 5 ml of sodium hydroxide (50 g/l) TS, 1.0 ml of alkaline potassiomercuric iodide TS, heat to boiling, and allow to stand; a yellow precipitate is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 6 volumes of 2-butanol R, 2 volumes of ammonia (~260 g/l) TS, and 2 volumes of ethylene glycol monomethyl ether R as the mobile phase. Apply separately to the plate 10 µml of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.25 g of Allopurinol with a mixture of 1.0 ml of diethylamine R and 9 ml of water, filter, and use the filtrate. For solution (B) use 0.05 mg of aminopyrazole-4-carboxamide hemisulfate RS per ml of ammonia (~260 g/l) TS. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.1 g of Allopurinol add 20 ml of sodium hydroxide (0.05 mol/l) VS and shake for 20 minutes. Then add 80 ml of hydrochloric acid (0.1 mol/l) VS and shake for 10 minutes. Dilute to 250 ml with hydrochloric acid (0.1 mol/l) VS, filter, and dilute 10 ml of the filtrate to 250 ml with the same acid. Measure the absorbance of a 1-cm layer at the maximum at about 250 nm against a solvent cell containing hydrochloric acid (0.1 mol/l) VS.

Calculate the percentage content of $C_5H_4N_4O$ using the absorptivity value of 56.3 ($A^{1\%}_{1\,cm}$ = 563).

AMPHOTERICINI B PULVIS AD INJECTIONEM
AMPHOTERICIN B POWDER FOR INJECTIONS

Description. A yellow to orange powder; odourless or almost odourless.

Category. Antifungal drug.

Storage. Amphotericin B powder for injections should be protected from light and stored at a temperature between 2 and 8 °C.

Labelling. The label should state whether any buffering agents and preservatives are added. Further, it should indicate that it is intended for intravenous or intrathecal administration, and that it should be protected from light. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Amphotericin B powder for injections is a sterile powder of amphotericin B mixed with sodium desoxycholate.

In water, it yields a colloidal dispersion.

Amphotericin B powder for injections may contain suitable buffers and preservatives. The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

Identity tests

A. Dissolve a quantity of the powder for injections equivalent to 25 mg of Amphotericin B in 5 ml of dimethyl sulfoxide R, add sufficient methanol R to produce 50 ml, and dilute 2 ml to 200 ml with methanol R. The absorption spectrum of the resulting solution, when observed between 300 nm and 450 nm, exhibits three maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance of a 1-cm layer at 362 nm to that at 381 nm is about 0.6; the ratio of the absorbance at 381 nm to that at 405 nm is about 0.9.

B. Dissolve a quantity of the powder for injections equivalent to 1 mg of Amphotericin B in 2 ml of dimethyl sulfoxide R and introduce 5 ml of phosphoric acid (~1440 g/l) TS to form a lower layer; a blue ring is immediately formed at the interface of the two liquids. Mix the two liquids; a strong blue colour is produced. Add 15 ml of water and mix; the colour of the solution changes to pale yellow.

Loss on drying. Dry the powder for injections to constant mass at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury); it loses not more than 80 mg/g.

pH value. pH of a suspension of the powder for injections containing 10 mg of Amphotericin B per ml of carbon-dioxide-free water R, 7.2–8.0.

Assay
Mix the contents of 10 containers and carry out the assay as described.

Triturate a quantity of the powder for injections equivalent to about 0.06g of Amphotericin B, accurately weighed, with dimethylformamide R and add, with shaking, sufficient dimethylformamide R to produce 100ml. Dilute 10ml to 100ml with dimethylformamide R and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Saccharomyces cerevisiae* (NCTC 10716, or ATCC 9763) as the test organism, culture medium Cm3 with a final pH of 6.1, sterile phosphate buffer, pH 10.5, TS1, an appropriate concentration of Amphotericin B (usually between 0.5 and 10.0µg/ml), and an incubation temperature of 29–33°C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 750µg/g, calculated with reference to the dried substance.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.0IU of endotoxin RS per mg.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure.

AMPICILLINI CAPSULAE
AMPICILLIN CAPSULES

Category. Antibacterial drug.

Storage. Ampicillin capsules should be kept in a tightly closed container and stored at a temperature not exceeding 25°C.

Labelling. The label should state whether the active ingredient is in the anhydrous form or is the trihydrate, and the quantity should be indicated in terms of the equivalent amount of ampicillin. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 250mg, 500mg.

Requirements

Comply with the monograph for "Capsules".

Ampicillin capsules contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{19}N_3O_4S$ stated on the label.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 65 volumes of acetone R, 10 volumes of water, 10 volumes of toluene R, and 2.5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 2 µl of each of the following two solutions. For solution (A) shake a quantity of the contents of the capsules equivalent to 50 mg of Ampicillin with 10 ml of a mixture of 4 volumes of acetone R and 1 volume of hydrochloric acid (0.1 mol/l) VS, filter, and use the clear filtrate. For solution (B) dissolve 25 mg of ampicillin RS in 5 ml of the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, spray lightly with triketohydrindene/ethanol TS, dry at 90 °C for 15 minutes, and examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. Shake a quantity of the contents of the capsules equivalent to 10 mg of Ampicillin with 3 ml of water and filter. To the filtrate add 0.1 g of hydroxylamine hydrochloride R and about 0.4 ml of sodium hydroxide (~80 g/l) TS and allow to stand for 5 minutes. Add 1.3 ml of hydrochloric acid (~70 g/l) TS and 0.5 ml of ferric chloride (25 g/l) TS; a violet-red to violet-brown colour is produced.

C. Shake a quantity of the contents of the capsules equivalent to 0.5 g of Ampicillin with 5 ml of water for 5 minutes, filter, wash the residue with ethanol (~750 g/l) TS, and dry it under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 1 hour. Place 2 mg of the residue in a test-tube, add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS, and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS, and mix; the solution is colourless to slightly pink. Immerse the test-tube for 1 minute in a water-bath; an orange-yellow colour is produced.

Loss on drying. Dry a quantity of the contents of the capsules equivalent to 0.1 g of the active ingredient at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours. For capsules containing anhydrous Ampicillin the loss is not more than 40 mg/g, and for capsules containing Ampicillin trihydrate the loss is not less than 100 mg/g and not more than 150 mg/g of the initial quantity taken.

Assay. To a quantity of the mixed contents of 20 capsules equivalent to about 0.12 g of Ampicillin, accurately weighed, add 400 ml of water and shake for 30 minutes. Dilute to 500 ml with water and filter. Transfer 10 ml of the filtrate to a 100-ml volumetric flask, add 10 ml of buffer borate, pH 9.0, TS and 1 ml of acetic anhydride/dioxan TS, allow to stand for 5 minutes at room temperature, and dilute to volume with water. Transfer two 2-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay, measure the absorbance of a 1-cm layer at the maximum at about 325 nm, against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{16}H_{19}N_3O_4S$ in the substance being examined by comparison with ampicillin RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.29 ± 0.02.

AMPICILLINI NATRICI PULVIS AD INJECTIONEM

AMPICILLIN SODIUM POWDER FOR INJECTIONS

Description. A white or almost white powder.

Category. Antibacterial drug.

Storage. Ampicillin sodium powder for injections should be protected from light and stored at a temperature not exceeding 25 °C. The reconstituted solution should be used immediately after preparation.

Labelling. The label should state the dose as the equivalent amount of anhydrous ampicillin. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg in vials.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Ampicillin sodium powder for injections is a sterile powder of ampicillin sodium.

The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

The container of Ampicillin sodium powder for injections contains not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{19}N_3O_4S$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ampicillin sodium RS or with the *reference spectrum* of ampicillin sodium.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 65 volumes of acetone R, 10 volumes of water, 10 volumes of toluene R, and 2.5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 2 µl of each of the following two solutions. For solution (A) shake a quantity of the powder for injections equivalent to 5 mg of Ampicillin sodium with 10 ml of a mixture of 4 volumes of acetone R and 1 volume of hydrochloric acid (0.1 mol/l) VS, filter, and use the clear filtrate. For solution (B) dissolve 25 mg of ampicillin sodium RS in 5 ml of the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, spray lightly with triketohydrindene/ethanol TS, dry at 90 °C for 15 minutes, and examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powder for injections equivalent to 2 mg of Ampicillin sodium in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place the same quantity of powder in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS, and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; a dark yellow colour is produced.

D. When tested for sodium as described under 2.1 General identification tests, the powder for injections yields the characteristic reactions. If reaction B is to be used, ignite a small quantity of the powder for injections and dissolve the residue in acetic acid (~60 g/l) TS.

Specific optical rotation. Use a solution containing a quantity of the powder for injections equivalent to 5 mg of Ampicillin sodium per ml in acetate standard buffer TS and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +260$ to $+290\,°$.

Clarity of solution. A freshly prepared solution of the powder for injections equivalent to 1 g of Ampicillin sodium in 10 ml of water is clear. A similar solution in 10 ml of hydrochloric acid (1 mol/l) VS is also clear.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using a quantity of the powder for injections equivalent to 0.5 g of Ampicillin sodium; the water content is not more than 20 mg/g.

pH value. pH of a solution containing a quantity of the powder for injections equivalent to 0.1 g of Ampicillin sodium per ml of carbon-dioxide-free water R, 8.0–10.0.

Assay

Mix the contents of 10 containers and carry out the assay as described.

Dissolve a quantity of the powder for injections equivalent to about 0.12 g of Ampicillin sodium, accurately weighed, in sufficient water to produce 500 ml. Transfer 10 ml of this solution to a 100-ml volumetric flask, add 10 ml of buffer borate, pH 9.0, TS and 1 ml of acetic anhydride/dioxan TS, allow to stand for 5 minutes at room temperature, and dilute to volume with water. Transfer two 2-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate as a percentage the amount of $C_{16}H_{19}N_3O_4S$ in the powder for injections by comparison with ampicillin RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.29 ± 0.02.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.15 IU of endotoxin RS per mg of ampicillin.

ARTEMETHERI CAPSULAE
ARTEMETHER CAPSULES

Category. Antimalarial drug.

Storage. Artemether capsules should be kept in a hermetically closed container and stored in a cool place.

Additional information. Available strengths: 40 mg, 50 mg.

Requirements

Comply with the monograph for "Capsules".

Artemether capsules contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{26}O_5$ stated on the label.

Identity tests

- Either tests A and B, or tests B, C, and D may be applied.

A. To a quantity of the contents of the capsules equivalent to 0.040 g of Artemether add 40 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the contents of the capsules equivalent to 0.08 g of Artemether add 40 ml of dehydrated ethanol R, shake to dissolve, and filter. Evaporate half of the filtrate to about 1 ml (keep the remaining filtrate for test D), add 0.10 g of potassium iodide R and heat; a yellow colour is produced.

D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R. For solution (A) shake a quantity of the contents of the capsules equivalent to about 20 mg of Artemether with 2 ml of acetone R, filter, and use the filtrate. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artemether per ml, solution (C) with the equivalent of about 0.025 mg of Artemether per ml, and solution (D) with the equivalent of about 0.10 mg of Artemether per ml. For solution (E) use 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

   Prepare the following solutions in the mobile phase. For solution (A) mix the contents of 20 capsules, shake a quantity equivalent to about 0.05 g

of Artemether, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of the mobile phase. For solution (B) use 10 mg of artemether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{16}H_{26}O_5$.

B. Mix the contents of 20 capsules and transfer a quantity equivalent to about 13 mg of Artemether, accurately weighed, to a 100-ml volumetric flask and dilute to volume with dehydrated ethanol R. Shake the flask for 15 minutes and filter, discarding the first 10 ml of the filtrate. Accurately measure 5 ml of the clear filtrate into a 50-ml volumetric flask and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 °C for 5 hours. Allow to cool to room temperature. For the blank use 5 ml of dehydrated ethanol R diluted with sufficient hydrochloric acid/ethanol (1 mol/l) VS to produce 50 ml.

Measure the absorbance of a 1-cm layer at the maximum at about 254 nm against a solvent cell containing the blank. Calculate the percentage content of $C_{16}H_{26}O_5$ in the capsules being examined by comparison with artemether RS, similarly and concurrently examined.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ARTEMETHERI COMPRESSI
ARTEMETHER TABLETS

Category. Antimalarial drug.

Additional information. Available strengths: 40 mg, 50 mg.

Requirements

Comply with the monograph for "Tablets".

Artemether tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{26}O_5$ stated on the label.

Identity tests

- Either tests A and B, or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to 0.040 g of Artemether add 40 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the powdered tablets equivalent to 0.08 g of Artemether add 40 ml of dehydrated ethanol R, shake to dissolve, and filter. Evaporate half of the filtrate to about 1 ml (keep the remaining filtrate for test D), add 0.10 g of potassium iodide, and heat; a yellow colour is produced.

D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R. For solution (A) shake a quantity of the powdered tablets equivalent to about 20 mg of Artemether with 2 ml of acetone R, filter, and use the filtrate. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artemether per ml, solution (C) with the equivalent of about 0.025 mg of Artemether per ml, and solution (D) with the equivalent of about 0.10 mg of Artemether per ml. For solution (E) use 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 0.05 g of Artemether, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness and dissolve the residue in 5 ml of the mobile phase. For solution (B) use 10 mg of artemether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{16}H_{26}O_5$.

B. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 13 mg of Artemether, accurately weighed, to a 100-ml volumetric flask and dilute to volume with dehydrated ethanol R. Shake the flask for 15 minutes and filter, discarding the first 10 ml of the filtrate. Accurately measure 5 ml of the clear filtrate into a 50-ml volumetric flask and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 °C for 5 hours. Allow to cool to room temperature. For the blank use 5 ml of dehydrated ethanol R diluted with sufficient hydrochloric acid/ethanol (1 mol/l) VS to produce 50 ml.

   Measure the absorbance of a 1-cm layer at the maximum at about 254 nm against a solvent cell containing the blank. Calculate the percentage content of $C_{16}H_{26}O_5$ in the tablets being examined by comparison with artemether RS, similarly and concurrently examined.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ARTEMETHERI INJECTIO
ARTEMETHER INJECTION

Description. A clear, colourless or almost colourless, oily solution.

Category. Antimalarial drug.

Storage. Artemether injection should be kept protected from light and stored in a cool place.

Labelling. The oil used in the formulation should be indicated.

Additional information. Strength in the current WHO Model list of essential medicines: 80 mg/ml in 1-ml ampoule; other available strengths: 40 mg/ml (paediatric formulation), 60 mg/ml, 100 mg/ml (adult formulation).

Artemether injection is normally intended for intramuscular administration.

Requirements

Complies with the monograph for "Parenteral preparations" and with 5.6 Test for extractable volume for parenteral preparations, 3.4 Test for bacterial endotoxins, *and* 5.7 Visual inspection of particulate matter in injectable preparations.

Definition. Artemether injection is a sterile solution of artemether in a suitable oil for injection.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization).

Artemether injection contains not less than **95.0%** and not more than **105.0%** of the amount of $C_{16}H_{26}O_5$ stated on the label.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. To a volume of the injection equivalent to 0.050 g of Artemether add 25 ml of acetone R, mix, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a volume of Artemether injection equivalent to about 30 mg of Artemether add 6 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- Either test A or test B may be applied.

A. *Note*: This test cannot be performed if arachis oil is present in the formulation.

Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than

twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R. For solution (A) dilute a volume of the injection with acetone R to obtain a concentration equivalent to 10 mg of Artemether per ml. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artemether per ml, solution (C) with the equivalent of about 0.025 mg of Artemether per ml, and solution (D) with the equivalent of about 0.10 mg of Artemether per ml. For solution (E) use 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in the mobile phase. For solution (A) dilute a volume of the injection to obtain a concentration equivalent to 10 mg of Artemether per ml, for solution (B) use 10 mg of artemether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{16}H_{26}O_5$.

B. Dilute an accurately measured volume of the injection equivalent to about 0.08 g with sufficient ethanol R to produce 100 ml. Dilute 5 ml of this solution with the same solvent to 50 ml and mix. Transfer a further 5 ml of the diluted solution to a 50-ml volumetric flask and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 ± 1 °C for 5 hours. Allow to cool to room temperature.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 254 nm. (*Note*: If arachis oil is used in the formulation of Artemether injection subtract the value of 0.025 from the absorbance value determined; the correction in absorbance value for other oils would have to be established.) Calculate the percentage content of $C_{16}H_{26}O_5$ in the formulation being examined by comparison with artemether RS, similarly and concurrently examined.

ARTEMISININI CAPSULAE
ARTEMISININ CAPSULES

Category. Antimalarial drug.

Storage. Artemisinin capsules should be kept in a cool place.

Additional information. Available strength: 250 mg.

Requirements

Comply with the monograph for "Capsules".

Artemisinin capsules contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{22}O_5$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. To a quantity of the contents of the capsules equivalent to 0.040 g of Artemisinin add 40 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemisinin RS or with the *reference spectrum* of artemisinin.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the contents of the capsules equivalent to 10 mg of Artemisinin add 20 ml of dehydrated ethanol R, shake to dissolve, filter, and evaporate to dryness. To half of the residue (keep the remaining residue for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, and 4 drops of starch TS; a violet colour is immediately produced.

D. Evaporate the remaining filtrate from test C to dryness on a water-bath. Dissolve the residue in 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). The mobile phases for gradient elution consist of a mixture of acetonitrile and water, using the conditions shown in the following table:

Time (min)	Mobile phase A (% v/v of acetonitrile)	Mobile phase B (% v/v of water)	Comment
0–17	60	40	Isocratic
17–30	60 ⇒ 100	40 ⇒ 0	Linear gradient
30–35	100 ⇒ 60	0 ⇒ 40	Return to initial conditions
35–45	60	40	Isocratic – re-equilibration

Prepare the following solutions. For solution (A) mix the contents of 20 capsules, shake a quantity equivalent to about 10 mg of Artemisinin, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

For solution (B) use 50 µg of Artemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution B (0.25%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution B.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R. For solution (A) take a quantity of the contents of the capsules equivalent to about 0.10 g of Artemisinin, add 10 ml of toluene R, shake vigorously, filter into a 10-ml volumetric flask, and dilute to volume with toluene R. Prepare similarly solution (B) containing the equivalent of about 0.05 mg of Artemisinin per ml, solution (C) the equivalent of about 0.025 mg of Artemisinin per ml, and solution (D) with the equivalent of about 0.10 mg of Artemisinin per ml. For solution (E) use 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/sulfuric acid TS, and heat the plate to 105 °C for 7 minutes. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase. For solution (A) mix the contents of 20 capsules, shake a quantity equivalent to about 1.0 mg of Artemisinin, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml. For solution (B) use 1.0 mg of artemisinin RS per ml.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{15}H_{22}O_5$.

B. Mix the contents of 20 capsules and transfer a quantity equivalent to about 0.05 g of Artemisinin, accurately weighed, to a 100-ml volumetric flask and dilute to volume with ethanol (~750 g/l) TS. Shake the flask, filter, and discard the first 20 ml of the filtrate. Dilute 10 ml of the filtrate to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $C_{15}H_{22}O_5$ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ARTEMISININI COMPRESSI
ARTEMISININ TABLETS

Category. Antimalarial drug.

Storage. Artemisinin tablets should be kept in a cool place.

Additional information. Available strength: 250 mg.

Requirements

Comply with the monograph for "Tablets".

Artemisinin tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{22}O_5$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to 0.040 g of Artemisinin add 40 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemisinin RS or with the *reference spectrum* of artemisinin.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the powdered tablets equivalent to 10 mg of Artemisinin add 20 ml of dehydrated ethanol R, shake to dissolve, filter, and evaporate to dryness. To half of the residue (keep the remaining residue for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, and 4 drops of starch TS; a violet colour is immediately produced.

D. Evaporate the remaining filtrate from test C to dryness on a water-bath. Dissolve the residue in 0.5 ml of dehydrated ethanol R, add about 0.5 ml

of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). The mobile phases for gradient elution consist of a mixture of acetonitrile and water, using the conditions shown in the following table:

Time (min)	Mobile phase A (% v/v of acetonitrile)	Mobile phase B (% v/v of water)	Comment
0–17	60	40	Isocratic
17–30	60 ⇒ 100	40 ⇒ 0	Linear gradient
30–35	100 ⇒ 60	0 ⇒ 40	Return to initial conditions
35–45	60	40	Isocratic – re-equilibration

Prepare the following solutions. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 10 mg of Artemisinin, accurately weighed, add 2 ml of acetone R, shake, and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of a mixture of 8 volumes of acetonitrile R and 2 volumes of water. For solution (B) use 50 µg of Artemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution B (0.25%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution B.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R. For solution (A) take a quantity of the powdered tablets equivalent to about 0.10 g of Artemisinin, add 10 ml of toluene R, shake vigorously, filter into a 10-ml volumetric flask, and dilute to volume with toluene R. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artemisinin per ml, solution (C) with the equivalent of about 0.025 mg of Artemisinin per ml, and solution (D) with the equivalent of about 0.10 mg of Artemisinin per ml. For solution (E) use 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/sulfuric acid TS, and heat the plate to 105 °C for 7 minutes. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 1.0 mg of Artemisinin, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml. For solution (B) use 1.0 mg of artemisinin RS per ml.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{15}H_{22}O_5$.

B. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.05 g of Artemisinin, accurately weighed, to a 100-ml volumetric flask and dilute to volume with ethanol (~750 g/l) TS. Shake the flask, filter, and discard the first 20 ml of the filtrate. Dilute 10 ml of the filtrate to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $C_{15}H_{22}O_5$ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ARTEMOTILI INJECTIO
ARTEMOTIL INJECTION

Description. A clear, colourless to slightly yellowish, oily solution.

Category. Antimalarial drug.

Storage. Artemotil injection should be kept protected from light.

Labelling. The oil used in the formulation should be indicated.

Additional information. Available strengths: 50 mg/ml (paediatric formulation), 75 mg/ml, 150 mg/ml (adult formulation).

Artemotil injection is normally intended for intramuscular administration.

Requirements

Complies with the monograph for "Parenteral preparations", and with 5.6 Test for extractable volume for parenteral preparations, 3.4 Test for bacterial endotoxins, and 5.7 Visual inspection of particulate matter in injectable preparations.

Definition. Artemotil injection is a sterile solution of artemotil in an oil suitable for injection.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization).

Artemotil injection contains not less than **95.0%** and not more than **105.0%** of the amount of $C_{17}H_{28}O_5$ stated on the label.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. To a volume of the injection equivalent to 0.050 g of Artemotil add 25 ml of acetone R, mix, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artemotil RS or with the *reference spectrum* of artemotil.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a volume of the injection equivalent to about 30 mg of Artemotil add 6 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R. For solution (A) dilute a volume of the injection with toluene R to obtain a concentration equivalent to 10 mg of Artemotil per ml. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artemotil per ml, solution (C) with the equivalent of about 0.025 mg of Artemotil per ml, and solution (D) with the equivalent of 0.10 mg of Artemotil per ml. For solution (E) use 0.10 mg of artemotil RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following solutions in acetonitrile R. For solution (A) dilute a volume of the injection to obtain a concentration equivalent to 10 mg of Artemotil per ml; for solution (B) use 10 mg of artemotil RS per ml; and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemotil per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{17}H_{28}O_5$.

ARTENIMOLI COMPRESSI
ARTENIMOL TABLETS

Category. Antimalarial drug.

Storage. Artenimol tablets should be kept in a cool place and protected from light.

Additional information. Available strength: 20 mg.

Requirements

Comply with the monograph for "Tablets".

Artenimol tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{24}O_5$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to 0.040 g of Artenimol add 40 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artenimol RS or with the *reference spectrum* of artenimol.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the powdered tablets equivalent to 10 mg of Artenimol add 20 ml of dehydrated ethanol R, shake to dissolve, filter, and evaporate to dryness. To half of the residue (keep the remaining residue for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, and 4 drops of starch TS; a violet colour is produced.

D. Evaporate the remaining filtrate from test C to dryness on a water-bath. Dissolve the residue in 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep reddish-brown colour is immediately produced.

Related substances

- Either test A or test B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase for gradient elution, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water for the first 17 minutes; then run a gradient, which should reach 100% acetonitrile within 13 minutes.

   Prepare the following solutions. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 10 mg of Artenimol, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of methanol R with sonication. For solution (B) dissolve 50 µg of Artenimol per ml in methanol R with sonication.

   For the system suitability test prepare solution (C) by dissolving 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in methanol R with sonication.

   Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

   Inject alternately 20 µl each of solutions A, B, and C.

   The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

   Measure the areas of the peak (twin-peak) responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the twin peak, is not greater than that

obtained with solution B (0.5%). Not more than one peak is greater than half the area of the twin peak obtained with solution B (0.25%). The sum of the areas of all the peaks, other than the twin peak, is not greater than twice the area of the twin peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the twin peak in the chromatogram obtained with solution B.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R. For solution (A) shake a quantity of the powdered tablets equivalent to about 20 mg of Artenimol, with 2 ml of acetone R, and filter. Use the filtrate. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artenimol per ml, solution (C) with the equivalent of about 0.025 mg of Artenimol per ml, and solution (D) with the equivalent of about 0.10 mg of Artenimol per ml. For solution (E) use 0.10 mg of artenimol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 1.0 mg of Artenimol, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml. For solution (B) use 1.0 mg of artenimol RS per ml.

For the system suitability test prepare solution (C) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak (twin-peak) responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{15}H_{24}O_5$.

B. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.05 g of Artenimol, accurately weighed, add sufficient ethanol (~750 g/l) TS to produce 100 ml, shake, and filter. Discard the initial 20 ml of the filtrate and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $C_{15}H_{24}O_5$ in the substance being tested by comparison with artenimol RS, similarly and concurrently examined.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ARTESUNATI COMPRESSI
ARTESUNATE TABLETS

Category. Antimalarial drug.

Storage. Artesunate tablets should be kept in a cool place.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg.

Requirements

Comply with the monograph for "Tablets".

Artesunate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{28}O_8$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to 0.050 g of Artesunate add 25 ml of acetone R, shake to dissolve, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artesunate RS or with the *reference spectrum* of artesunate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 5 volumes of ethyl acetate R and 95 volumes of toluene R as the mobile phase. Apply separately to the plate 2 µl of the following 2 solutions in toluene R. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.10 mg of Artesunate in dehydrated ethanol R, filter, and evaporate. Dissolve the residue in 1.0 ml of toluene R. For solution (B) use 0.10 mg of artesunate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/methanol TS, and heat the plate to 120 °C for 5 minutes. Examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powdered tablets equivalent to 0.1 g of Artesunate add 40 ml of dehydrated ethanol R, shake to dissolve, and filter. To half of the filtrate (keep the remaining filtrate for test D) add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a light redviolet colour is produced.

D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish, add 1 drop of vanillin/sulfuric acid TS1, a reddish-brown colour is produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given below under Assay method A.

Inject alternately 20 µl each of solutions A and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution C (1.0%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.5%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (2.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 48 volumes of light petroleum R1, 36 volumes of ethyl acetate R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 3 solutions in dichloromethane R. For solution (A) shake a quantity of the powdered tablets equivalent to about 10 mg of Artesunate with 2 ml of dichloromethane R, filter, and use the filtrate. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artesunate per ml, and solution (C) with the equivalent of about 0.025 mg of Artesunate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (1.0%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%).

Assay

- Either method A or method B may be applied.

A. Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (12.5 cm × 3.5 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of equal volumes of acetonitrile R and buffer pH 3.0 (dissolve 1.36 g of potassium dihydrogen

phosphate R in 1000 ml of water and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).

Prepare the following solutions in acetonitrile R. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 4.0 mg of Artesunate, accurately weighed, with 2 ml of acetone R, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml. For solution (B) use 4.0 mg of artesunate RS per ml, and for solution (C) dilute solution A to obtain a concentration equivalent to 0.04 mg of Artesunate per ml.

Operate with a flow rate of 0.6 ml per minute. Maintain the column temperature at 30 °C and use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{19}H_{28}O_8$.

B. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.5 g of Artesunate, accurately weighed, add 50 ml of neutralized ethanol TS, shake thoroughly, filter, and discard about 10 ml of the initial filtrate. Titrate 25 ml of the filtrate with sodium hydroxide (0.05 mol/l) VS, using 2 drops of phenolphthalein/ethanol TS as indicator.

Each ml of sodium hydroxide (0.05 mol/l) VS is equivalent to 19.22 mg of $C_{19}H_{28}O_8$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ATROPINI SULFATIS COMPRESSI
ATROPINE SULFATE TABLETS

Category. Antispasmodic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 1 mg.

Requirements

Comply with the monograph for "Tablets".

Atropine sulfate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ stated on the label.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 5 volumes of chloroform R, 4 volumes of acetone R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 10 mg of Atropine sulfate with 2 ml of ethanol (~750 g/l) TS, centrifuge, and use the supernatant liquid. For solution (B) dissolve 25 mg of atropine sulfate RS in 5 ml of ethanol (~750 g/l) TS. After removing the plate from the chromatographic chamber, heat it at 105 °C for 20 minutes, allow to cool, and spray with potassium iodobismuthate TS2. Examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. Triturate a quantity of the powdered tablets equivalent to 1 mg of Atropine sulfate with 1 drop of ammonia (~260 g/l) TS, add 2 ml of chloroform R, and triturate again thoroughly. Filter the chloroform layer and evaporate. To the residue add about 0.2 ml of fuming nitric acid R and evaporate to dryness on a water-bath; a yellow residue is obtained. To the cooled residue add 2 ml of acetone R and about 0.2 ml of potassium hydroxide/methanol TS; a deep violet colour is produced.

C. A filtered solution of the powdered tablets in water yields the reactions described under 2.1 General identification tests as characteristic of sulfates.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 2.5 mg of Atropine sulfate, accurately weighed, into a 50-ml volumetric flask, add 30 ml of water, shake well, dilute to volume, and filter. Discard the first few ml of filtrate and use the successive clear filtrate as the *test solution*. For the *reference solution* use 25 mg of atropine sulfate RS, accurately weighed and previously dried to constant mass at 120 °C, dissolve in sufficient water to produce 25 ml, and mix well. Dilute 5 ml of this solution to 100 ml with water (= 50 µg of anhydrous atropine sulfate per ml). Transfer 2 ml of each of the *test solution* and the *reference solution* to two 60-ml separating funnels containing 10 ml of chloroform R. Add 2 ml of bromocresol green TS1, shake for 2 minutes, and allow to stand until two layers are formed.

Measure the absorbance of the chloroform layers of the *test solution* and the *reference solution* at the maximum at about 420 nm against a solvent cell containing chloroform R.

Calculate the amount in mg of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the sample being examined using the following formula: $1.027(M/10)(A_u/A_s)$, in which M is the mass in mg of atropine sulfate RS in the *reference solution* and A_u and A_s are the absorbances for the *test solution* and the *reference solution*, respectively.

Uniformity of content. Individually transfer 10 powdered tablets to 10 separate stoppered test-tubes, to each add 6 ml of water, accurately measured, shake thoroughly for 30 minutes, centrifuge, and use the clear solution as the *test solution*. For the *reference solution* weigh accurately 25 mg of atropine sulfate RS, previously dried to constant mass at 120 °C, dissolve in sufficient water to produce 25 ml, and mix well. Dilute 5 ml of this solution to 100 ml with water (= 50 µg of anhydrous atropine sulfate per ml). Transfer 2 ml of each of the *solutions to be examined* and the *reference solution* to two 60-ml separating funnels containing 10 ml of chloroform R. Add 2 ml of bromocresol green TS1, shake for 2 minutes, and allow to stand until two layers are formed.

Measure the absorbance of the chloroform layers of the *solutions to be examined* and the *reference solution* at the maximum at about 420 nm against a solvent cell containing chloroform R. Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in mg, using the following formula: $1.027(M/10)(A_u/A_s)$, in which M is the mass in mg of atropine sulfate RS in the *reference solution* and A_u and A_s are the absorbances for the *solutions examined* and the *reference solution*, respectively.

The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

BENZYLPENICILLINI KALICI PULVIS AD INJECTIONEM

BENZYLPENICILLIN POTASSIUM POWDER FOR INJECTIONS

Description. A white or almost white, crystalline powder.

Category. Antibacterial drug.

Storage. Unless otherwise recommended by the manufacturer, the reconstituted solution should be used within 24 hours when stored at a temperature not exceeding 20 °C or within 7 days (14 days if a buffering agent is present) when stored at a temperature between 2 and 8 °C.

Labelling. The designation on the container should state the nature of the buffering agent. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 600 mg and 3 g of Benzylpenicillin.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Benzylpenicillin potassium powder for injections is a sterile powder of benzylpenicillin potassium.

Benzylpenicillin potassium powder for injections usually contains a suitable buffering agent. The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

The container of Benzylpenicillin potassium powder for injections contains not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{17}KN_2O_4S$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from benzylpenicillin potassium RS or with the *reference spectrum* of benzylpenicillin potassium.

B. Dissolve a quantity of the powder for injections equivalent to 0.1 g of Benzylpenicillin potassium in sufficient phosphate buffer, pH 7.0, (0.067 mol/l) TS to produce 100 ml. Dilute 10 ml with the same reagent to produce 100 ml (*solution A*). To 10 ml of *solution A* add 0.5 ml of a solution prepared by diluting 1 ml of penicillinase TS to 10 ml with water and allow to stand at 30 °C for 10 minutes (*solution B*). Place 5 ml of each of *solutions A* and *B* in two separate test-tubes and add to each tube 10 ml of acetate buffer, pH 4.6, TS and 5 ml of iodine (0.0005 mol/l) VS. After mixing the contents of each tube add 0.1 ml of starch TS; the mixture obtained with *solution A* is blue and that with *solution B* remains colourless.

C. To a quantity of the powder for injections equivalent to 2 mg of Benzylpenicillin potassium in a test-tube add 1 drop of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless.

Place a further similar quantity of the powder for injections in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS, and mix; the solution is brownish yellow. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

D. Ignite a small quantity of the powder for injections, dissolve the residue in water, and filter; on addition of 2 ml of sodium hydroxide (~80 g/l) TS to the filtrate it yields the reaction described under 2.1 General identification tests as characteristic of potassium.

Clarity and colour of solution. A solution of the powder for injections equivalent to 0.2 g of Benzylpenicillin potassium in 10 ml of carbon-dioxide-free water R is clear and colourless. (Keep this solution for the "pH value".)

Loss on drying. Dry the powder for injections to constant mass at 105 °C; it loses not more than 10 mg/g.

pH value. pH of the solution prepared above for the test of clarity and colour, 5.5–7.5.

Assay

Mix the contents of 10 containers and carry out the assay as described.

Dissolve a quantity of the powder equivalent to about 50 mg of Benzylpenicillin potassium, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{16}H_{17}KN_2O_4S$ in the powder for injections by comparison with benzylpenicillin sodium RS similarly and concurrently examined, taking into account that each mg of benzylpenicillin sodium RS ($C_{16}H_{17}N_2NaO_4S$) is equivalent to 1.045 mg of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$). In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.62 ± 0.03.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

CARBAMAZEPINI COMPRESSI
CARBAMAZEPINE TABLETS

Category. Antiepileptic agent.

Storage. Carbamazepine tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg, 200 mg.

Requirements

Comply with the monograph for "Tablets".

Carbamazepine tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{12}N_2O$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

Transfer a quantity of the powdered tablets equivalent to about 0.5 g of Carbamazepine to a 50-ml beaker, add 10 ml of warm acetone R, and shake. Filter while still warm, evaporate the filtrate to dryness on a water-bath, and dry at 80 °C. Dissolve in acetone R, allow to recrystallize, and use the crystals for the following tests.

A. Carry out the examination with the crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from carbamazepine RS or with the *reference spectrum* of carbamazepine.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To about 0.1 g of the crystals add about 2 ml of nitric acid (~1000 g/l) TS and heat in a water-bath for 1 minute; an orange colour is produced.

D. Melting temperature of the crystals, about 189 °C.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 95 volumes of toluene R and 5 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of the following 3 solutions. For solu-

tion (A) shake a quantity of the powdered tablets equivalent to about 0.20 g of Carbamazepine with three 10-ml quantities of chloroform R and filter. Evaporate the combined filtrates to dryness and dissolve the residue in 10 ml of chloroform R. For solution (B) use 20 mg of carbamazepine RS per ml of chloroform R. For solution (C) use 0.06 mg of iminodibenzyl R per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air for 15 minutes, spray with potassium dichromate TS3, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.06 g of Carbamazepine add 25 ml of ethanol (~750 g/l) TS and boil for a few minutes. Stir the hot mixture in a closed flask for 10 minutes and filter through a sintered glass filter. Wash the flask with ethanol (~750 g/l) TS, filter, and dilute the cooled filtrate with sufficient ethanol (~750 g/l) TS to produce 100 ml. Dilute 5 ml to 250 ml with the same solvent. Measure the absorbance of a 1-cm layer at the maximum at about 285 nm against a solvent cell containing ethanol (~750 g/l) TS.

Calculate the percentage content of $C_{15}H_{12}N_2O$ using the absorptivity value of 49.0 ($A^{1\%}_{1\,cm} = 490$).

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

CHLOROQUINI PHOSPHATIS COMPRESSI
CHLOROQUINE PHOSPHATE TABLETS

Category. Antiamoebic drug; antimalarial drug.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg, 150 mg.

Requirements

Comply with the monograph for "Tablets".

Chloroquine phosphate tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_{18}H_{26}ClN_3{,}2H_3PO_4$ stated on the label.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution D.

B. Shake a quantity of the powdered tablets equivalent to 5 mg of Chloroquine phosphate with 1 ml of water and filter. To the filtrate add 1 ml of silver nitrate (40 g/l) TS; a yellow precipitate is produced. To a portion of the precipitate add a few drops of nitric acid (~130 g/l) TS; a clear solution is obtained. To another portion of the precipitate add a few drops of ammonia (~100 g/l) TS and shake; the yellow precipitate dissolves but a small amount of white precipitate remains.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of chloroform R, 4 volumes of cyclohexane R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 2 µl of each of the following four solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.5 g of Chloroquine phosphate with 50 ml of water for 30 minutes, centrifuge, and use the supernatant liquid; if necessary, filter through a glass-fibre paper. For solution (B) dilute 5 ml of solution A to 100 ml with water, and for solution (C) dilute 25 ml of solution B to 50 ml with water. For solution (D) dissolve 8 mg of chloroquine sulfate RS in 1 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B, and not more than one such spot is more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.5 g of Chloroquine phosphate, accurately weighed, to a separating funnel, add 20 ml of sodium hydroxide (1 mol/l) VS, and extract with four quantities, each of 25 ml, of chloroform R. Filter each extract through a glass-fibre paper washed with chloroform R and kept moistened with the solvent. Evaporate the combined chloroform extracts to about 10 ml, add 40 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 25.79 mg of $C_{18}H_{26}ClN_3,2H_3PO_4$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

CHLOROQUINI SULFATIS COMPRESSI
CHLOROQUINE SULFATE TABLETS

Category. Antiamoebic drug; antimalarial drug.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg, 150 mg.

Requirements

Comply with the monograph for "Tablets".

Chloroquine sulfate tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_{18}H_{26}ClN_3,H_2SO_4$ stated on the label.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution D.

B. Shake a quantity of the powdered tablets equivalent to 0.1 g of Chloroquine sulfate with 10 ml of water and 1 ml of hydrochloric acid (~70 g/l) TS, and filter. To the filtrate add 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of chloroform R, 4 volumes of cyclohexane R, and 1 volume of diethylamine R as the mobile phase. Apply separately to the plate 2 µl of each of the following four solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.4 g of Chloroquine sulfate with 50 ml of water for 30 minutes, centrifuge, and use the supernatant liquid; if necessary, filter through a glass-fibre paper and use the clear filtrate. For solution (B) dilute 5 ml of solution A to 100 ml with water, and for solution (C) dilute 25 ml of solution B to 50 ml with water. For solution (D) dissolve 8 mg of chloroquine sulfate RS in 1 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B, and not more than one such spot is more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.5 g of Chloroquine sulfate, accurately weighed, to a separating funnel, add 20 ml of sodium hydroxide (1 mol/l) VS, and extract with four quantities, each of 25 ml, of chloroform R. Filter each extract through a glass-fibre paper previously washed with chloroform R and kept moistened with the solvent. Evaporate the combined chloroform extracts to about 10 ml, add 40 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.90 mg of $C_{18}H_{26}ClN_3,H_2SO_4$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

CHLORPHENAMINI HYDROGENOMALEATIS COMPRESSI

CHLORPHENAMINE HYDROGEN MALEATE TABLETS

Other name. Chlorpheniramine hydrogen maleate tablets.

Category. Antiallergic drug.

Storage. Chlorphenamine hydrogen maleate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 4 mg.

Requirements

Comply with the monograph for "Tablets".

Chlorphenamine hydrogen maleate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{19}ClN_2,C_4H_4O_4$ stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. Triturate a quantity of the powdered tablets equivalent to 25 mg of Chlorphenamine hydrogen maleate with 20 ml of hydrochloric acid (~70 g/l) TS. Separately dissolve 25 mg of Chlorphenamine hydrogen maleate RS in 20 ml of hydrochloric acid (~70 g/l) TS. To each solution add sufficient sodium hydroxide (~80 g/l) TS to render them alkaline to a pH of about 11, then extract with two 50-ml portions of hexane R. Collect the extracts in beakers and evaporate to dryness. Carry out the examination with the residues as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum of the sample being examined is concordant with the spectrum obtained from Chlorphenamine hydrogen maleate RS.

B. See the test described below under "Related substances". Under an ultraviolet light the two principal spots obtained with solution A correspond in position, appearance, and intensity with those obtained with solution B. After spraying, the principal spot obtained with solution A corresponds to that obtained with solution B.

C. To a quantity of the powdered tablets equivalent to 40 mg of Chlorphenamine hydrogen maleate add about 20 ml of water, warm the mixture, and filter. To the filtrate add 10 ml of a saturated solution of trinitrophenol R in water and warm on a water-bath for 5 minutes; a precipitate is produced. Filter, wash the precipitate with water, collect the precipitate, and dry it at 105 °C for 1 hour; melting behaviour, about 196 °C with decomposition.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and heating the coated plate at 105 °C for 30 minutes. Use as the mobile phase a mixture of 5 volumes of ethyl acetate R, 3 volumes of methanol R, and 2 volumes of acetic acid (~60 g/l) TS. Apply separately to the plate 2 µl of each of the following four solutions. For solution (A) extract a quantity of the powdered tablets equivalent to 5 mg of Chlorphenamine hydrogen maleate with chloroform R, filter, evaporate the filtrate to dryness, and dissolve the residue in 1 ml of chloroform R. For solution (B) dissolve 25 mg of chlorphenamine hydrogen maleate RS in 5 ml of chloroform R. For solution (C) extract a quantity of the powdered tablets equivalent to 50 mg of Chlorphenamine hydrogen maleate with chloroform R, filter, evaporate the filtrate to dryness, and dissolve the residue in 1 ml of chloroform R. For solution (D) dilute 0.2 ml of solution C to 100 ml with chloroform R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm) for identification purposes, then spray it with potassium iodobismuthate TS2.

Any spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powder equivalent to about 3 mg of Chlorphenamine hydrogen maleate, accurately weighed, with 20 ml of sulfuric acid (0.05 mol/l) VS for 5 minutes. Add 20 ml of hexane R, shake carefully, and filter the acid layer into a second separator. Extract the hexane layer with two quantities, each of 10 ml, of sulfuric acid (0.05 mol/l) VS, filtering each acid layer into the second separator, and wash the filter with sulfuric acid (0.05 mol/l) VS. Add sodium hydroxide (1 mol/l) VS to the acid extracts and washings to make the solution just alkaline to litmus paper R, add 2 ml in excess, and extract with two quantities, each of 50 ml, of hexane R. Wash each hexane extract with the same 20 ml of water, and extract in succession with 20 ml, 20 ml, and 5 ml of sulfuric acid (0.25 mol/l) VS. Dilute the combined acid extracts to 50 ml with sulfuric acid (0.25 mol/l) VS and dilute 10 ml to 25 ml with the same acid.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 265 nm. Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$, using the absorptivity value of 21.2 ($A^{1\%}_{1\,cm} = 212$).

Uniformity of content. Individually transfer 10 powdered tablets to 10 separate stoppered test-tubes, and shake with 20 ml of sulfuric acid (0.05 mol/l) VS for 5 minutes. Add 20 ml of hexane R, shake carefully, and filter the acid layer into a second separator. Extract the hexane layer with two quantities, each of 10 ml, of sulfuric acid (0.05 mol/l) VS, filtering each acid layer into the second separator, and wash the filter with sulfuric acid (0.05 mol/l) VS. Add sodium hydroxide (1 mol/l) VS to the acid extracts and washings to make the solution just alkaline to litmus paper R, add 2 ml in excess, and extract with two quantities, each of 50 ml, of hexane R. Wash each hexane extract with the same 20 ml of water, and extract in succession with 20 ml, 20 ml, and 5 ml of sulfuric acid (0.25 mol/l) VS. Dilute the combined acid extracts to 50 ml with sulfuric acid (0.25 mol/l) VS, dilute 10 ml to 25 ml with the same acid.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 265 nm. Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$, using the absorptivity value of 21.2 ($A^{1\%}_{1\,cm} = 212$).

The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

CLOXACILLINI NATRICI CAPSULAE
CLOXACILLIN SODIUM CAPSULES

Category. Antibacterial drug.

Storage. Cloxacillin sodium capsules should be kept in a tightly closed container.

Labelling. The designation on the container should state the quantity of Cloxacillin sodium in terms of the equivalent amount of cloxacillin. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: the equivalent of 500 mg of cloxacillin.

Requirements

Comply with the monograph for "Capsules".

Cloxacillin sodium capsules contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{18}ClN_3O_5S$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from cloxacillin sodium RS or with the *reference spectrum* of cloxacillin sodium.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silanized silica gel R3 as the coating substance and a mixture of 30 volumes of acetone R and 70 volumes of a solution containing 154 g/l of ammonium acetate R, the pH of which has been adjusted to 5.0 with glacial acetic acid R, as the mobile phase. Apply separately to the plate 1 µl of each of the following three solutions. For solution (A) shake a quantity of the contents of the capsules equivalent to 0.25 g of Cloxacillin sodium with 50 ml of water, filter, and use the clear filtrate. For solution (B) dissolve 25 mg of cloxacillin sodium RS in 5 ml of water. For solution (C) dissolve 25 mg of each of cloxacillin sodium RS, dicloxacillin sodium RS, and flucloxacillin sodium RS together in 5 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air, and expose it to the vapour of iodine R until spots appear. Examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows three distinctly separated spots.

C. Place a quantity of the contents of the capsules equivalent to 2 mg of Cloxacillin sodium into a test-tube, and add 2 mg of disodium chromotropate R and 2 ml of sulfuric acid (~1760 g/l) TS. Immerse the tube in a suitable bath at 150 °C for 3–4 minutes; a red-violet colour is produced.

D. Ignite a quantity of the contents of the capsules equivalent to 20 mg of Cloxacillin sodium and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a filtered solution containing a quantity of the contents of the capsules equivalent to 10 mg of Cloxacillin sodium per ml, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +163$ to $+ 172\,°$.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using a quantity of the contents of the capsules equivalent to 0.25 g of Cloxacillin sodium; the water content is not more than 50 mg/g.

pH value. pH of a filtered solution containing a quantity of the contents of the capsules equivalent to 0.10 g of Cloxacillin sodium per ml of carbon-dioxide-free water R, 5.0–7.0.

Assay. Mix the contents of 20 capsules and transfer a quantity equivalent to about 0.25 g of Cloxacillin sodium, accurately weighed, into a glass-stoppered flask. Add 70 ml of water, shake the flask for 15 minutes, and dilute with sufficient water to produce 500 ml. Filter and dilute 10 ml of the filtrate to 100 ml with water. Transfer two 2.0-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10.0 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 343 nm against a solvent cell containing a mixture of 2.0 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{19}H_{18}ClN_3O_5S$ in the substance being examined by comparison with cloxacillin sodium RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.40 ± 0.02.

CLOXACILLINI NATRICI PULVIS AD INJECTIONEM

CLOXACILLIN SODIUM POWDER FOR INJECTIONS

Description. A white, crystalline powder.

Category. Antibacterial drug.

Storage. Cloxacillin sodium powder for injections should be protected from light.

Labelling. The designation on the container should state the quantity of Cloxacillin sodium in terms of the equivalent amount of cloxacillin. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: the equivalent of 500 mg of cloxacillin in vials.

Cloxacillin sodium powder for injections is hygroscopic.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Cloxacillin sodium powder for injections is a sterile powder of cloxacillin sodium.

The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

The container of Cloxacillin sodium powder for injections contains not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{17}ClN_3NaO_5S$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from cloxacillin sodium RS or with the *reference spectrum* of cloxacillin sodium.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silanized silica gel R3 as the coating substance and a mixture of 30

volumes of acetone R and 70 volumes of a solution containing 154 g/l of ammonium acetate R, the pH of which has been adjusted to 5.0 with glacial acetic acid R, as the mobile phase. Apply separately to the plate 1 µl of each of the following three solutions. For solution (A) shake a quantity of the powder for injections equivalent to 0.25 g of Cloxacillin sodium with 50 ml of water, filter, and use the clear filtrate. For solution (B) dissolve 25 mg of cloxacillin sodium RS in 5 ml of water, and for solution (C) dissolve 25 mg of each of cloxacillin sodium RS, dicloxacillin sodium RS, and flucloxacillin sodium RS together in 5 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air and expose it to the vapour of iodine R until spots appear. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows three distinctly separated spots.

C. Place a quantity of the powder for injections equivalent to 2 mg of Cloxacillin sodium in a test-tube and add 2 mg of disodium chromotropate R and 2 ml of sulfuric acid (~1760 g/l) TS. Immerse the tube in a suitable bath at 150 °C for 3–4 minutes; a red-violet colour is produced.

D. Ignite a quantity of the powder for injections equivalent to 20 mg of Cloxacillin sodium and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Specific optical rotation. Use a solution containing a quantity of the powder for injections equivalent to 10 mg of Cloxacillin sodium per ml, and calculate with reference to the anhydrous substance; $[\alpha]_D^{20\,°C} = +163$ to $+173°$.

Clarity and colour of solution. A solution of the powder for injections equivalent to 0.2 g of Cloxacillin sodium in 10 ml of carbon-dioxide-free water R is clear and colourless. (Keep this solution for the "pH value".)

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using a quantity of the powder for injections equivalent to 0.25 g of Cloxacillin sodium; the water content is not less than 35 mg/g and not more than 45 mg/g.

pH value. pH of the solution prepared above for the test of clarity and colour, 5.0–7.0.

Assay

Mix the contents of 10 containers and carry out the assay as described.

Transfer a quantity of the powder for injections equivalent to about 0.1 g of Cloxacillin, accurately weighed, to a 500-ml flask and dilute to volume with water. Dilute 25 ml to 100 ml with water. Transfer two 2-ml aliquots of this solution into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 343 nm against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{19}H_{17}ClN_3NaO_5S$ in the substance being examined by comparison with cloxacillin sodium RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.40 ± 0.02.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.40 IU of endotoxin RS per mg of cloxacillin.

CODEINI PHOSPHATIS COMPRESSI
CODEINE PHOSPHATE TABLETS

Category. Opioid analgesic, antidiarrhoeal, antitussive.

Additional information. Strength in the current WHO Model list of essential medicines: 30 mg. Codeine phosphate tablets are prepared either from the hemihydrate or the sesquihydrate.

Requirements

Comply with the monograph for "Tablets".

Codeine phosphate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{18}H_{21}NO_3,H_3PO_4$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.1 g of Codeine phosphate add 15 ml of water and 5 ml of sulfuric acid (~100 g/l) TS, and

allow to stand for 1 hour. Filter, if necessary, and wash any undissolved residue with a few ml of water. Render the filtrate alkaline with ammonia (~100 g/l) TS and extract with several small portions of chloroform R. Evaporate the combined extracts to dryness on a water-bath and dry the residue at 80 °C for 4 hours. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from the *reference spectrum* of codeine phosphate.

Shake a quantity of the powdered tablets equivalent to about 0.06 g of codeine phosphate with four 10-ml portions of ethanol (~750 g/l) TS and filter. Evaporate the combined filtrate to dryness on a water-bath and use the residue for the following tests.

B. Dissolve 10 mg of the residue in 5 ml of sulfuric acid (~1760 g/l) TS, add 1 drop of ferric chloride (25 g/l) TS and, if necessary, heat gently; a violet-blue colour is produced. Add a few drops of nitric acid (~130 g/l) TS; the colour changes to dark red.

C. Dissolve 20 mg of the residue in 1.0 ml of water and add 1 drop of ferric chloride (25 g/l) TS; a precipitate is formed but no blue tinge is observed in the solution (distinction from morphine).

D. Dissolve 10 mg of the residue in 2.0 ml of carbon-dioxide-free water R and add a few drops of silver nitrate (40 g/l) TS; a yellow precipitate is produced. Divide the solution with the precipitate into 2 portions. To 1 portion add a few drops of nitric acid (~130 g/l) TS; the precipitate dissolves to a clear solution. To the other portion add a few drops of ammonia (~100 g/l) TS and shake well; again the precipitate dissolves to a clear solution.

Assay. Weigh and powder 25 tablets. To a quantity of the powder equivalent to about 0.2 g of Codeine phosphate add sufficient water to produce a thin suspension, then add 20 ml of a mixture of 1 part of sulfuric acid (~100 g/l) TS and 3 parts of water. Shake occasionally over a period of 2 hours, then allow to stand for 12 hours, and filter. Add 5 ml of sodium hydroxide (~80 g/l) TS and extract with successive quantities of 15 ml, 10 ml, 10 ml, and 5 ml of chloroform R. Evaporate the combined extracts to dryness on a water-bath, dissolve the residue in 20 ml of glacial acetic acid R1, and titrate with perchloric acid (0.05 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.05 mol/l) VS is equivalent to 19.87 mg of $C_{18}H_{21}NO_3,H_3PO_4$.

COLCHICINI COMPRESSI
COLCHICINE TABLETS

Category. Drug used for the treatment of gout.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Requirements

Comply with the monograph for "Tablets".

Colchicine tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{22}H_{25}NO_6$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Triturate a quantity of the powdered tablets equivalent to about 20 mg of Colchicine with 20 ml of water. Allow the solids to settle and filter the supernatant liquid into a separatory funnel. Shake with 30 ml of chloroform R. Evaporate the chloroform layer to dryness using mild heat. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from colchicine RS or with the *reference spectrum* of colchicine.

B. The absorption spectrum of the solution obtained in the "Assay", when observed between 230 nm and 380 nm, exhibits two maxima at about 243 nm and 350 nm. The ratio of the absorbance at 243 nm to that at 350 nm is between 1.80 and 2.00.

C. Suspend a quantity of the powdered tablets in 1.5 ml of ethanol (~750 g/l) TS and filter. Place a few drops of the filtrate on a porcelain dish and evaporate to dryness on a water-bath. Mix the residue with 3 drops of sulfuric acid (~1760 g/l) TS; a lemon yellow colour is produced. Add 1 drop of nitric acid (~130 g/l) TS; the colour changes to greenish blue, turning rapidly to reddish, and finally becoming yellowish. Following this add about 0.5 ml of sodium hydroxide (~200 g/l) TS; the colour turns to red.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using a suitable aluminium oxide R as the coating substance, containing a substance that fluoresces at about 254 nm, and a mixture of 125 volumes of chloroform R, 100 volumes of acetone R, and 2 volumes of

ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 2 ml of each of the following 2 µ solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 5 mg of Colchicine with 5 ml of chloroform R, filter, and evaporate the filtrate to dryness in a current of air. Dissolve the residue as completely as possible in about 0.1 ml of ethanol (~750 g/l) TS. Allow to settle and use the supernatant liquid. For solution (B) dilute 1 volume of solution A to 20 volumes with ethanol (~750 g/l) TS. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay
The operations described below must be carried out in subdued light.

Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.5 mg of Colchicine add 10 ml of dehydrated ethanol R and shake for 30 minutes. Centrifuge, separate, and wash the residue with dehydrated ethanol R. Combine the extract and washings and dilute to 50 ml with the same solvent. Measure the absorbance of a 1-cm layer at the maximum at about 350 nm against a solvent cell containing dehydrated ethanol R.

Calculate the percentage content of $C_{22}H_{25}NO_6$ using the absorptivity value of 42.5 ($A^{1\%}_{1\,cm}$ = 425).

Uniformity of content
The operations described below must be carried out in subdued light.

Place 1 tablet in a centrifuge tube and add 10 ml of dehydrated ethanol R. Crush the tablet to a fine powder, shake for 30 minutes, centrifuge, and wash the residue with dehydrated ethanol R. Combine the extract and washings and dilute to produce a solution of 0.01 mg/ml of dehydrated ethanol R. Measure the absorbance of a 1-cm layer at the maximum at about 350 nm against a solvent cell containing dehydrated ethanol R.

Calculate the tablet content of $C_{22}H_{25}NO_6$ in mg using the absorptivity value of 42.5 ($A^{1\%}_{1\,cm}$ = 425). The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

DAPSONI COMPRESSI
DAPSONE TABLETS

Category. Antileprosy drug.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg, 100 mg.

The tablets may be coloured.

Requirements

Comply with the monograph for "Tablets".

Dapsone tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_{12}H_{12}N_2O_2S$ stated on the label.

Identity tests

- Either tests A and B or tests B and C may be applied.

A. To a quantity of the powdered tablets equivalent to 0.1 g of Dapsone add 50 ml of methanol R, shake, and filter. Dilute 0.5 ml of the filtrate to 200 ml with methanol R. The absorption spectrum of the resulting solution, when observed between 230 nm and 350 nm, exhibits maxima at about 260 nm and 295 nm; the absorbances of a 1-cm layer at these maximum wavelengths are about 0.36 and 0.6, respectively.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Shake a quantity of the powdered tablets equivalent to 0.05 g of Dapsone with 5 ml of warm acetone R, filter, evaporate the filtrate, and dry at 105 °C for 30 minutes. Dissolve the residue in 2 ml of hydrochloric acid (~70 g/l) TS, cool in ice, and add 4 ml of sodium nitrite (10 g/l) TS. Allow to stand for 2 minutes then pour the mixture into 2 ml of freshly prepared 2-naphthol TS1 containing 1 g of sodium acetate R; an orange-red precipitate is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R1 as the coating substance and a mixture of 8 volumes of toluene R and 4 volumes of acetone R as the mobile phase. Apply separately to the plate 1 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 10 mg of Dapsone

with 10 ml of methanol R, filter, and use the clear filtrate. For solution (B) dissolve 5 mg of dapsone RS in 5 ml of methanol R. Further apply 10 µl of the following three solutions. For solution (C) shake a quantity of the powdered tablets equivalent to 0.1 g of Dapsone with 10 ml of methanol R, filter, and use the clear filtrate. For solution (D) dilute 1 ml of solution C to 100 ml with methanol R, and for solution (E) dilute 1 ml of solution D to 5 ml with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air and spray first with sodium nitrite/hydrochloric acid TS and then, while still damp, with *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS, and examine the chromatogram in daylight.

Any spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D, and not more than two such spots are more intense than that obtained with solution E.

Assay. Weigh and powder 20 tablets. Dissolve a quantity of the powder equivalent to about 0.25 g of Dapsone, accurately weighed, in a mixture of 15 ml of water and 15 ml of hydrochloric acid (~70 g/l) TS. Carry out the assay as described under 2.7 Nitrite titration, titrating with sodium nitrite (0.1 mol/l) VS.

Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

DEXAMETHASONI COMPRESSI
DEXAMETHASONE TABLETS

Category. Adrenal hormone.

Additional information. Strength in the current WHO Model list of essential medicines: 500 µg, 4 mg.

Requirements

Comply with the monograph for "Tablets".

Dexamethasone tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{22}H_{29}FO_5$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 20 mg of Dexamethasone add 50 ml of chloroform R and shake for 30 minutes. Filter, evaporate the filtrate to dryness, and dry the residue at 105 °C for 2 hours. Use the residue for the following tests.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dexamethasone RS or with the *reference spectrum* of dexamethasone.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 9 volumes of acetone R and 1 volume of formamide R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of 3 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the residue per ml, (B) 2.5 mg of dexamethasone RS per ml, and (C) a mixture of equal volumes of solutions A and B. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, and spray the hot plate with sulfuric acid/ethanol TS. Heat at 120 °C for a further 10 minutes, allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. A single spot is obtained with solution C.

C. Transfer a solution containing about 0.2 mg of the residue in 2.0 ml of ethanol (~750 g/l) TS to a stoppered test-tube, add 10 ml of phenylhydrazine/sulfuric acid TS, mix, heat in a water-bath at 60 °C for 20 minutes, and cool immediately. The absorbance of a 1-cm layer at the maximum at about 423 nm is not less than 0.40.

D. Transfer about 10 mg to a porcelain crucible, add 45 mg of magnesium oxide R, and ignite until an almost white residue is obtained. Allow to cool, add 2.0 ml of water, 0.05 ml of phenolphthalein/ethanol TS, and 1.0 ml of hydrochloric acid (~70 g/l) TS. Filter, to the filtrate add a freshly prepared mixture of 0.10 ml of sodium alzarinsulfonate (1 g/l) TS and 0.10 ml of zirconyl nitrate TS, mix, and allow to stand for 5 minutes. Repeat the test without the substance being examined. A yellow colour is produced in the solution of the substance being examined and the reagent blank turns red.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 4.0 mg of Dexamethasone, add 15 ml of water, and shake with four quantities, each of 25 ml, of chloroform R. Filter the chloroform layers through cotton wool, previously washed with chloroform R, and add sufficient chloroform R to produce 200 ml. Transfer 10 ml of the resulting solution to a glass-stoppered 50-ml conical flask, carefully evaporate to dryness, and dissolve the residue in 20 ml of dehydrated ethanol R. Add 2.0 ml of blue tetrazolium/ethanol TS and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner.

Calculate the percentage content of $C_{22}H_{29}FO_5$ by comparison with dexamethasone RS, similarly and concurrently examined.

Uniformity of content

To 1 tablet add 15 ml of water and shake until the tablet is completely disintegrated. Extract with four quantities of 1 ml of chloroform R, filter the chloroform layers through cotton wool, previously washed with chloroform R, and add sufficient chloroform R to produce 50 ml. Transfer a volume of this solution, equivalent to about 200 µg of Dexamethasone, to a glass-stoppered 50-ml conical flask, carefully evaporate to dryness, and dissolve the residue in 20 ml of dehydrated ethanol R. Add 2.0 ml of blue tetrazolium/ethanol TS and displace the air in the flask with oxygen-free nitrogen R. Immediately add 2.0 ml of tetramethylammonium hydroxide/ethanol TS and again displace the air with oxygen-free nitrogen R. Stopper the flask, mix the contents by gentle swirling, and allow to stand for 1 hour in a water-bath at 30 °C. Cool rapidly, add sufficient aldehyde-free ethanol (~750 g/l) TS to produce 25 ml, and mix. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing a solution prepared by treating 10 ml of aldehyde-free ethanol (~750 g/l) TS in a similar manner.

Calculate the tablet content of $C_{22}H_{29}FO_5$ in mg by comparison with dexamethasone RS, similarly and concurrently examined. The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

DIETHYLCARBAMAZINI DIHYDROGENOCITRATIS COMPRESSI

DIETHYLCARBAMAZINE DIHYDROGEN CITRATE TABLETS

Category. Antifilarial drug.

Storage. Diethylcarbamazine dihydrogen citrate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg.

Requirements

Comply with the monograph for "Tablets".

Diethylcarbamazine dihydrogen citrate tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_{10}H_{21}N_3O,C_6H_8O_7$ stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

A. To a quantity of the powdered tablets equivalent to 0.15 g of Diethylcarbamazine dihydrogen citrate add 15 ml of ethanol (~750 g/l) TS, shake for 5 minutes, filter, and evaporate the filtrate to dryness. To the residue add 10 ml of sodium hydroxide (~80 g/l) TS and extract with three 10-ml quantities of chloroform R. Dry the combined extracts over anhydrous sodium sulfate R, filter, evaporate the filtrates, and carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diethylcarbamazine dihydrogen citrate RS similarly treated or with the *reference spectrum* of diethylcarbamazine.

B. To a quantity of the powdered tablets equivalent to 0.2 g of Diethylcarbamazine dihydrogen citrate add 10 ml of water, shake, and filter. Transfer the filtrate to a separatory funnel, add 1 ml of sodium hydroxide (~400 g/l) TS, and extract with 20 ml, 15 ml, and 10 ml of chloroform R. Keep the aqueous layer for test C. Evaporate the combined chloroform extracts on a water-bath and, towards the end, by drying with the aid of a current of air. Dissolve the oily residue in 10 ml of ethyl acetate R, warming the mixture to 50 °C, and pour it into 2 ml of a solution containing 1 g of maleic acid R in 10 ml of acetone R, warming again to 50 °C. Cool, rub the sides of the tube with a glass rod to induce crystallization, collect the white precipitate on a

sintered-glass filter, wash twice with 1 ml of acetone R and once with 5 ml of ethyl acetate R, and dry in a desiccator; melting point, 126–128 °C.

C. Filter the aqueous layer from test B. Add 1 drop of phenolphthalein/ethanol TS and neutralize with sulfuric acid (~100 g/l) TS. Then add 2 ml of mercuric sulfate TS, heat to boiling and add, drop by drop, potassium permanganate (10 g/l) TS; the violet colour is discharged and a white precipitate is produced.

***N*-Methylpiperazine.** Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 6 volumes of ethanol (~750 g/l) TS, 3 volumes of glacial acetic acid R, and 1 volume of water as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.5 g of Diethylcarbamazine dihydrogen citrate with 10 ml of methanol R, filter, and use the clear filtrate. For solution (B) dissolve 5 mg of *N*-methylpiperazine R in 100 ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a mixture of 3 volumes of platinic chloride (60 g/l) TS, 97 volumes of water, and 100 volumes of potassium iodide (60 g/l) TS, and examine the chromatogram in daylight.

The spot obtained with solution B is more intense than any spot, corresponding in position and appearance, obtained with solution A.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.75 g of Diethylcarbamazine dihydrogen citrate, accurately weighed, to a separatory funnel, add 10 ml of water and 10 ml of sodium hydroxide (~200 g/l) TS, and shake to dissolve. Extract with four 25-ml quantities of chloroform R, washing each extract with the same two 20-ml quantities of water, and with a third quantity if the second is alkaline to phenolphthalein/ethanol TS. Extract the combined chloroform extracts with 25 ml of sulfuric acid (0.05 mol/l) VS and then with 15 ml and 10 ml of water. Combine the acid and water extracts, warm to remove the chloroform, cool, and back-titrate the excess of acid with sodium hydroxide (0.1 mol/l) VS using bromocresol green/ethanol TS as indicator.

Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 39.14 mg of $C_{10}H_{21}N_3O,C_6H_8O_7$.

DILOXANIDI FUROATIS COMPRESSI
DILOXANIDE FUROATE TABLETS

Category. Antiamoebic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Requirements

Comply with the monograph for "Tablets".

Diloxanide furoate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{14}H_{11}Cl_2NO_4$ stated on the label.

Identity tests

To a quantity of the powdered tablets equivalent to about 0.2 g of Diloxanide furoate add 20 ml of dichloromethane R and shake. Filter, evaporate the filtrate to dryness, and use the dried residue for the following tests.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from diloxanide furoate RS or with the *reference spectrum* of diloxanide furoate.

B. Melting temperature of the residue, about 115 °C.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 96 volumes of dichloromethane R and 4 volumes of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.5 g of Diloxanide furoate with 5 ml of chloroform R, centrifuge, and use the supernatant liquid. For solution (B) dilute 1 volume of solution A to 20 volumes of chloroform R, further dilute 1 volume of this solution to 20 volumes with the same solvent. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.04 g of Diloxanide furoate add 150 ml of ethanol (~750 g/l) TS and

shake for 30 minutes. Add sufficient ethanol (~750 g/l) TS to produce 200 ml, mix, and filter. Dilute 10 ml of the filtrate to 250 ml with the same solvent. Measure the absorbance of a 1-cm layer at the maximum at about 258 nm against a solvent cell containing ethanol (~750 g/l) TS.

Calculate the percentage content of $C_{14}H_{11}Cl_2NO_4$ using the absorptivity value of 70.5 ($A^{1\%}_{1\,cm} = 705$).

DOXYCYCLINI HYCLATIS COMPRESSI
DOXYCYCLINE HYCLATE TABLETS

Category. Antibacterial.

Storage. Doxycycline hyclate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg of doxycycline.

Requirements

Comply with the monograph for "Tablets".

Doxycycline hyclate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{22}H_{24}N_2O_8$ stated on the label, if Assay method A is applied.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance. Adjust the pH of a solution of 0.1 g of disodium edetate R per ml to 9.0 with sodium hydroxide (~400 g/l) TS, and spray this evenly onto the plate. Allow the plate to dry in a horizontal position for not less than 1 hour. Just before use, dry the plate in an oven at 110 °C for 1 hour. Use a mixture of 59 volumes of dichloromethane R, 35 volumes of methanol R, and 6 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of the following 3 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 5 mg of Doxycycline hyclate with 5 ml of methanol R, filter, dilute the filtrate to 10 ml with the same solvent, and use the resulting solution. For solution (B) dissolve 5 mg of doxycycline hyclate RS in methanol R and dilute

to 10 ml with the same solvent. For solution (C) dissolve 5 mg of doxycycline hyclate RS and 5 mg of tetracycline hydrochloride RS in methanol R, and dilute to 10 ml with the same solvent. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and examine the chromatogram in ultraviolet light (365 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows two clearly separated spots.

B. To a quantity of the powdered tablets equivalent to about 5 mg of Doxycycline hyclate add about 2 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced.

To a quantity of the powdered tablets equivalent to about 0.1 g of Doxycycline hyclate add 10 ml of water, filter, and use the filtrate for the following tests.

C. To 2.0 ml of the filtrate add 1 drop of ferric chloride (25 g/l) TS; a dark red-brown colour is produced.

D. To 1.0 ml of the filtrate add 5 drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is formed which dissolves in 1.0 ml of ammonia (~100 g/l) TS.

Light-absorbing impurities. To a quantity of the powdered tablets equivalent to about 0.10 g of Doxycycline hyclate add 10 ml of a mixture of 1 volume of hydrochloric acid (1 mol/l) VS and 99 volumes of methanol R, shake, and filter, discarding the first 2 ml of filtrate. Measure the absorbance of a 1-cm layer at 490 nm; the absorbance does not exceed 0.2.

Assay

- Either method A or method B may be applied.

A. Weigh and powder 20 tablets. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of styrene-divinylbenzene copolymer (8–10 µm). As the mobile phase, use a solution prepared as follows: transfer 60.0 g of *tert*-butanol R with the aid of 200 ml of water to a 1000-ml volumetric flask. Add 400 ml of buffer borate, pH 8.0, TS, 50 ml of a solution of 10 mg of tetrabutylammonium hydrogen sulfate R per ml adjusted to pH 8.0 with sodium hydroxide (~80 g/l) TS, and 20 ml of sodium edetate (20 g/l) TS adjusted to pH 8.0 with sodium hydroxide (~80 g/l) TS. Dilute to 1000 ml with water.

Prepare the following solutions in hydrochloric acid (0.01 mol/l) VS: for solution (A) use a quantity of the powdered tablets sufficient to give 0.80 mg of Doxycycline hyclate per ml; solution (B) 0.80 mg of doxycycline hyclate RS per ml; solution (C) 0.80 mg of 6-epidoxycycline hydrochloride RS per ml; solution (D) 0.80 mg of metacycline hydrochloride RS per ml; for solution (E) mix 4.0 ml of solution B with 1.5 ml of solution C and 1.0 ml of solution D, and dilute to 25 ml with hydrochloric acid (0.01 mol/l) VS; and for solution (F) mix 2.0 ml of solution C and 2.0 ml of solution D and dilute to 100 ml with hydrochloric acid (0.01 mol/l) VS.

Operate with a flow rate of about 0.9 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Inject 20 µl of solution E. The test is not valid unless the resolution between the first peak (metacycline) and the second peak (6-epidoxycycline) is not less than 1.25, and the resolution between the second peak and the third peak (doxycycline) is not less than 2.0. If necessary, adjust the *tert*-butanol R content in the mobile phase. The test is not valid unless the symmetry factor for the third peak is not more than 1.25. If necessary adjust the integrator parameters.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{22}H_{24}N_2O_8$ in the tablets, taking into account the declared content of $C_{22}H_{24}N_2O_8$ in doxycycline hyclate RS.

B. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 50 mg, accurately weighed, add 50 ml of dimethylformamide R and shake for 1 hour. Centrifuge, and carry out the assay with the supernatant liquid as described under 3.1 Microbiological assay of antibiotics, using *Bacillus cereus* (NCTC 10320 or ATCC 11778) as the test organism, culture medium Cm10 with a final pH of 6.6, potassium dihydrogen phosphate (13.6 g/l) TS as the buffer, an appropriate concentration of Doxycycline (usually between 0.2 and 2.0 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105%. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the content stated on the label expressed in mg, with 870 IU being equivalent to 1 mg of doxycycline.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

EPHEDRINI SULFATIS INJECTIO
EPHEDRINE SULFATE INJECTION

Description. A clear, colourless solution.

Category. Anti-asthmatic drug.

Storage. Ephedrine sulfate injection should be protected from light.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg/ml.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Ephedrine sulfate injection is a sterile solution of ephedrine sulfate in water for injections.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization).

Ephedrine sulfate injection contains not less than **95.0%** and not more than **105.0%** of the amount of $(C_{10}H_{15}NO)_2,H_2SO_4$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

To a volume of the injection equivalent to 0.1 g of Ephedrine sulfate add 5 ml of ethanol (~750 g/l) TS and evaporate to dryness on a water-bath with the aid of a stream of air. Use the residue for tests A and C.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ephedrine sulfate RS similarly treated or with the *reference spectrum* of ephedrine sulfate.

B. Measure the optical rotation of the injection; it is levorotatory.

C. Dissolve 10 mg of the residue in 1 ml of water and add 0.1 ml of copper(II) sulfate (80 g/l) TS, followed by 2 ml of sodium hydroxide (~80 g/l) TS; a violet colour is produced. To this solution add 2 ml of 1-butanol R and shake; a reddish violet colour is produced in the butanol layer.

D. The injection yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

pH value. pH of the injection, 4.5–7.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 80 volumes of 2-propanol R, 15 volumes of ammonia (~260 g/l) TS, and 5 volumes of chloroform R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) dilute a volume of the injection equivalent to 0.1 g of Ephedrine sulfate to 5 ml with methanol R, and for solution (B) dilute 0.5 ml of solution A to 100 ml with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a mixture of 0.2 g of triketohydrindene hydrate R dissolved in 95 ml of 1-butanol R and 5 ml of acetic acid (~120 g/l) TS, and heat to 110 °C for 5 minutes. Examine the chromatogram in daylight.

Any secondary spot obtained with solution A is not more intense than that obtained with solution B. Disregard any spot of lighter colour than the background.

Assay. Transfer an accurately measured volume of the injection equivalent to about 0.25 g of Ephedrine sulfate to a separator, dilute, if necessary, with water to a volume of about 10 ml, add 3 g of sodium chloride R to saturate the solution, then add 5 ml of sodium hydroxide (1 mol/l) VS, and extract four times, each with 25 ml of chloroform R. Wash the combined chloroform extracts with 10 ml of a saturated solution of sodium chloride R, and filter through purified cotton saturated with chloroform R. Shake the aqueous wash solution with 10 ml of chloroform R and add it to the main chloroform extract. Add 0.25 ml of methyl red/ethanol TS and titrate with perchloric acid/dioxan (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid/dioxan (0.1 mol/l) VS is equivalent to 21.43 mg of $(C_{10}H_{15}NO)_2,H_2SO_4$.

ERGOMETRINI HYDROGENOMALEATIS INJECTIO

ERGOMETRINE HYDROGEN MALEATE INJECTION

Description. A colourless to faintly yellow solution.

Category. Oxytocic.

Storage. Ergometrine hydrogen maleate injection should be kept in a single-dose container, protected from light, and stored in a cool place.

Labelling. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 200 µg/ml.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Ergometrine hydrogen maleate injection is a sterile solution of ergometrine hydrogen maleate in water for injections.

Ergometrine hydrogen maleate may contain a suitable stabilizer. The solution is sterilized by "Heating in an autoclave" or by another suitable method (see 5.8 Methods of sterilization).

Ergometrine hydrogen maleate injection contains not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ stated on the label.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution E.

B. To a volume of the injection equivalent to 0.1 mg of Ergometrine hydrogen maleate, add 0.5 ml of water and 2 ml of 4-dimethylaminobenzaldehyde TS1; a blue colour is slowly produced.

pH value. pH of the injection, 2.7–3.5.

Related substances. Carry out the test protected from light as described under 1.14.1 Thin-layer chromatography, using a suspension of silica gel R1 in sodium hydroxide (0.1 mol/l) VS as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of the following five solutions. For solution (A) evaporate a volume of the injection equivalent to 1 mg of Ergometrine hydrogen maleate to dryness at 20 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) and dissolve the residue in 0.25 ml of methanol R. Prepare solutions (B), (C), (D), and (E) in methanol R containing 0.1 mg/ml, 0.2 mg/ml, 0.4 mg/ml, and 4 mg/ml, respectively, of ergometrine hydrogen maleate RS. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet

light (365 nm) and in daylight after spraying with 4-dimethylaminobenzaldehyde TS2.

Assess the intensity of every spot, other than the principal spot, obtained with solution A by reference to the spots obtained with solutions B, C, and D; the total of intensities so assessed does not exceed 10% of the intensity of the principal spot. In addition, no single spot, other than the principal spot, obtained with solution A is more intense than that obtained with solution B.

Assay
The solutions must be protected from light throughout the assay.

Dilute an accurately measured volume of the injection with sufficient water to produce a concentration of about 0.04 mg of Ergometrine hydrogen maleate per ml. To 3 ml add 6 ml of 4-dimethylaminobenzaldehyde TS1, mix, cool to room temperature, and allow to stand for 30 minutes.

Measure the absorbance of a 1-cm layer at the maximum at about 545 nm against a solvent cell containing a reagent blank. Calculate the amount in mg of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the sample being examined by comparison with ergometrine hydrogen maleate RS.

ERGOMETRINI HYDROGENOMALEATIS COMPRESSI

ERGOMETRINE HYDROGEN MALEATE TABLETS

Category. Oxytocic.

Storage. Ergometrine hydrogen maleate tablets should be kept in a tightly closed container.

Labelling. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 200 µg.

Requirements

Comply with the monograph for "Tablets".

Ergometrine hydrogen maleate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ stated on the label.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution E.

B. Extract a quantity of the powdered tablets equivalent to 2 mg of Ergometrine hydrogen maleate with 20 ml of water, filter, and wash the residue with sufficient water to produce 20 ml; the solution has a blue fluorescence. To 2 ml add 4 ml of 4-dimethylaminobenzaldehyde TS1; a blue colour is slowly produced.

Related substances. Carry out the test protected from direct light as described under 1.14.1 Thin-layer chromatography, using a suspension of silica gel R1 in sodium hydroxide (0.1 mol/l) VS as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 5 µl of each of the following five solutions. For solution (A) triturate a quantity of the powdered tablets equivalent to 1 mg of Ergometrine hydrogen maleate with 0.2 ml of domiphen bromide (10 g/l) TS, add 2 ml of methanol R, centrifuge, and remove the supernatant liquid. Extract the residue with two further quantities, each of 1 ml, of methanol R. Evaporate the combined extracts to dryness at 20 °C under reduced pressure (0.6 kPa or 5 mm of mercury), and dissolve the residue in 0.25 ml of methanol R; centrifuge if necessary. Solutions (B), (C), (D), and (E) are solutions in methanol R containing 0.1 mg per ml, 0.2 mg per ml, 0.4 mg per ml, and 4 mg per ml, respectively, of ergometrine hydrogen maleate RS. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm).

Assess the intensity of each spot, other than the principal spot, obtained with solution A by reference to the spots obtained with solutions B, C, and D; the total of the intensities so assessed does not exceed 10% of the intensity of the principal spot. In addition, no single spot, other than the principal spot, obtained with solution A is more intense than the spot obtained with solution B.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powder equivalent to about 2 mg of Ergometrine hydrogen maleate, accurately weighed, with 50 ml of tartaric acid (10 g/l) TS for 30 minutes, centrifuge, and use the supernatant liquid. Dilute a suitable volume to produce a solution containing 0.040 mg per ml of ergometrine hydrogen maleate. To 3 ml add 6 ml of 4-dimethylaminobenzaldehyde TS1, mix, cool to room temperature, and allow to stand for 30 minutes.

Measure the absorbance of a 1-cm layer at the maximum at about 545 nm against a solvent cell containing a reagent blank composed of 6 ml of 4-dimethylaminobenzaldehyde TS1 and 3 ml of water. Calculate the amount in mg of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the sample being examined by comparison with a solution containing 0.04 mg/ml of ergometrine hydrogen maleate RS.

Uniformity of content. Individually transfer 10 powdered tablets to 10 separate stoppered test-tubes, add 10 ml of tartaric acid (10 g/l) TS, shake for 30 minutes, and centrifuge. Dilute a suitable volume to produce a solution containing 0.040 mg per ml of ergometrine hydrogen maleate. To 3 ml of a clear solution add 6 ml of 4 dimethylaminobenzaldehyde TS1, mix, cool to room temperature, and allow to stand for 30 minutes.

Measure the absorbance of a 1-cm layer at the maximum at about 545 nm against a solvent cell containing a reagent blank composed of 6 ml of 4-dimethylaminobenzaldehyde TS1 and 3 ml of water. Calculate the amount in mg of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the sample being examined by comparison with a solution containing 0.04 mg/ml of ergometrine hydrogen maleate RS.

The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

ERYTHROMYCINI ETHYLSUCCINATIS COMPRESSI

ERYTHROMYCIN ETHYLSUCCINATE TABLETS

Category. Antibacterial.

Storage. Erythromycin ethylsuccinate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg of erythromycin.

Requirements

Comply with the monograph for "Tablets".

Identity tests

- Either test A alone or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 0.25 g of Erythromycin ethylsuccinate add 20 ml of chloroform R and shake. Filter, evaporate the filtrate to dryness, and use the dried residue for tests A, C, and D.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from erythromycin ethylsuccinate RS or with the *reference spectrum* of erythromycin ethylsuccinate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 85 volumes of methanol R and 15 volumes of chloroform R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 30 mg of Erythromycin ethylsuccinate with 10 ml of methanol R by mechanical means for 30 minutes. Centrifuge a portion of this mixture and use the clear supernatant liquid. For solution (B) use 3 mg of erythromycin ethylsuccinate RS per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and spray with a mixture of 90 volumes of dehydrated ethanol R, 5 volumes of anisaldehyde R, and 5 volumes of sulfuric acid (~1760 g/l) TS. Heat the plate at 100 °C for 10 minutes and examine the chromatogram in daylight.

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To 5 mg of the residue add about 2 ml of sulfuric acid (~1760 g/l) TS and shake gently; a reddish brown colour is produced.

D. Dissolve about 3 mg of the residue in 2.0 ml of acetone R and add about 2 ml of hydrochloric acid (~420 g/l) TS; an orange colour is produced, which changes to orange-red and finally to violet-red. Add 2.0 ml of chloroform R and shake; the chloroform layer turns to blue.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.1 g (100 000 IU) of erythromycin, accurately weighed, add sufficient methanol R to produce 100 ml, shake, and allow the sediment to settle. Carefully transfer 40 ml of the clear solution to a 100-ml volumetric flask, dilute to volume with sterile phosphate buffer, pH 8.0, TS1 or TS2, and allow to stand, protected from light, for 5 hours at 20–25 °C. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS1 or TS2, an appropriate concentration of Erythromycin (usually between 5 and 15 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95%

and not more than 105%. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 110.0% of the content stated on the label, expressed in mg, with 1000 IU being equivalent to 1 mg of erythromycin.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ERYTHROMYCINI STEARATIS COMPRESSI
ERYTHROMYCIN STEARATE TABLETS

Category. Antibacterial.

Storage. Erythromycin stearate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg of erythromycin.

Requirements

Comply with the monograph for "Tablets".

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.2 g of Erythromycin stearate add 20 ml of water and shake. Decant the supernatant liquid and discard. Add 10 ml of methanol R to the residue, shake, filter, and evaporate to dryness. Carry out the examination with the dried residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from erythromycin stearate RS or with the *reference spectrum* of erythromycin stearate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 85 volumes of methanol R and 15 volumes of chloroform R as the mobile phase. Apply separately to the plate 20 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.05 g of Erythromycin stearate with 10 ml of methanol R by mechanical means for 30 minutes. Centrifuge a portion of this mixture and use the clear

supernatant liquid. For solution (B) use 5 mg of erythromycin stearate RS per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and spray with dichlorofluorescein TS. Heat the plate at 100 °C for 10 minutes and examine the chromatogram in ultraviolet light (365 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Next, spray the plate with a mixture of 90 volumes of dehydrated ethanol R, 5 volumes of anisaldehyde R, and 5 volumes of sulfuric acid (~1760 g/l) TS. Heat the plate at 100 °C for 10 minutes and examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powdered tablets equivalent to about 10 mg of Erythromycin stearate add 2.0 ml of acetone R and about 2 ml of hydrochloric acid (~420 g/l) TS and shake; a pale orange colour is produced, which changes to red or violet-red. Add 2.0 ml of chloroform R and shake; the chloroform layer acquires a violet colour.

D. Shake a quantity of the powdered tablets equivalent to about 0.1 g of Erythromycin stearate with 10 ml of chloroform R, filter, and evaporate the filtrate to dryness on a water-bath. Gently heat the residue with 10 ml of water and 5 ml of hydrochloric acid (~70 g/l) TS until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer, and heat it with 3.0 ml of sodium hydroxide (0.1 mol/l) VS. Allow to cool; the solution sets to a gel. Add 10 ml of hot water, shake, heat the mixture for 2–3 minutes and shake again; the solution froths. To 1.0 ml of the resulting solution add 2.0 ml of calcium chloride (55 g/l) TS; a granular precipitate is produced, which is insoluble in hydrochloric acid (~250 g/l) TS.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.1 g (100 000 IU) of erythromycin, accurately weighed, add sufficient methanol R to produce 100 ml, shake, and allow the sediment to settle. Carefully transfer 40 ml of the clear solution to a 100-ml volumetric flask, dilute to volume with sterile phosphate buffer, pH 8.0, TS1 or TS2, and allow to stand, protected from light, for 5 hours at 20–25 °C. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS1 or TS2, an appropriate concentration of Erythromycin (usually between 5 and 15 IU per ml), and an incubation temperature of 35–39 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95%

and not more than 105%. The upper fiducial limit of error is not less than 95.0% and the lower fiducial limit of error is not more than 110.0% of the content stated on the label, expressed in mg, with 1000 IU being equivalent to 1 mg of erythromycin.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ETHAMBUTOLI HYDROCHLORIDI COMPRESSI
ETHAMBUTOL HYDROCHLORIDE TABLETS

Category. Antituberculosis drug.

Additional information. Strength in the current WHO Model list of essential medicines: 100–400 mg.

Requirements

Comply with the monograph for "Tablets".

Ethambutol hydrochloride tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{10}H_{24}N_2O_2$, 2HCl stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

To a quantity of the powdered tablets equivalent to about 0.1 g of Ethambutol hydrochloride add 10 ml of methanol R and shake. Filter the extract and evaporate to dryness. Use the residue for tests A and C.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ethambutol hydrochloride RS or with the *reference spectrum* of ethambutol hydrochloride.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 100 volumes of methanol R and 1.5 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 10 mg of Ethambutol hydrochloride with 10 ml of water, filter, and use the filtrate. For solution (B) use 1.0 mg of ethambutol hydrochloride RS

per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and expose it to the vapour of iodine R until spots appear. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. The residue yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

Aminobutanol. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 55 volumes of ethyl acetate R, 35 volumes of glacial acetic acid R, 5 volumes of hydrochloric acid (~420 g/l) TS, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.5 g of Ethambutol hydrochloride with 10 ml of methanol R for 5 minutes, filter, and use the filtrate. For solution (B) use 0.5 mg of aminobutanol R per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, heat at 105 °C for 5 minutes, cool, spray with triketohydrindene/cadmium TS, and heat again at 90 °C for 5 minutes. Examine the chromatogram in daylight.

Any spot corresponding to aminobutanol obtained with solution A is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.2 g of Ethambutol hydrochloride add 10 ml of sodium hydroxide (~80 g/l) TS and extract with 5 portions, each of 20 ml, of chloroform R. Evaporate the combined extracts to a volume of about 25 ml, filter, and add 100 ml of glacial acetic acid R1. Add 1-naphtholbenzein/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 13.86 mg of $C_{10}H_{24}N_2O_2$, 2HCl.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

GLYCERYLIS TRINITRATIS COMPRESSI
GLYCERYL TRINITRATE TABLETS

Other name. Nitroglycerin tablets.

Category. Antianginal drug.

Storage. Glyceryl trinitrate tablets should be kept in a tightly closed container, preferably made of glass, with a screw closure lined with aluminium or tin foil, protected from light, and stored at a temperature not exceeding 20 °C. To prevent loss of potency, the tablets should be kept in the original container. Each container should hold not more than 100 tablets. After each use the container should be closed immediately.

Labelling. The designation on the container should state that the tablets are for sublingual use. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 500 µg.

CAUTION: Undiluted glyceryl trinitrate can explode by percussion or excessive heat. Avoid keeping isolated small amounts.

Requirements

Comply with the monograph for "Tablets".

Definition. Glyceryl trinitrate tablets are tablets for sublingual use.

Glyceryl trinitrate tablets contain not less than **80.0%** and not more than **120.0%** of the amount of $C_3H_5N_3O_9$ stated on the label.

Identity tests

A. Shake a quantity of the powdered tablets equivalent to 0.50 mg of Glyceryl trinitrate with 5 ml of dehydrated ethanol R, filter, and evaporate the filtrate to dryness using a stream of air. To the residue add 3–4 drops of sodium hydroxide (~80 g/l) TS and 3 ml of ferrous sulfate (15 g/l) TS and shake; a greenish brown precipitate is produced.

B. Extract a quantity of the powdered tablets equivalent to 5 mg of Glyceryl trinitrate with 3 ml of ethanol (~750 g/l) TS and filter. To the filtrate add carefully 1 ml of diphenylamine/sulfuric acid TS in a manner to form a lower layer; a dark blue colour is produced at the interface of the two layers.

Test for the absence of decomposition. Shake a quantity of the powdered tablets equivalent to 0.50 mg of Glyceryl trinitrate with 5 ml of water and filter. To the filtrate add 0.1 g of potassium iodide R, 2 drops of sulfuric acid (~100 g/l) TS, and 1 ml of starch TS; the liquid remains colourless. Add 1 ml of sodium hydroxide (~80 g/l) TS and heat gently to boiling. Cool and add 3 ml of sulfuric acid (~100 g/l) TS; a dark blue colour is immediately produced.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 1 mg of Glyceryl trinitrate, accurately weighed, add 4.5 ml of glacial acetic acid R and 0.5 ml of water, shake for 1 hour, and centrifuge. For the *reference solution,* dissolve 133.5 mg of potassium nitrate R, previously dried at 105 °C, in sufficient water to produce 50 ml. Transfer 10 ml to a volumetric flask and add sufficient glacial acetic acid R to produce 100 ml. To 1 ml of each of the clear *sample* and *reference solutions* add 2 ml of phenoldisulfonic acid TS, mix, and allow to stand for 15 minutes. Then add 8 ml of water, mix well, allow to cool, and add slowly with swirling 10 ml of ammonia (~260 g/l) TS. Cool and dilute to 20 ml with water.

Measure the absorbances of both solutions at the maximum at about 405 nm against a solvent cell containing a reagent blank. Calculate the amount in mg of $C_3H_5N_3O_9$ in the sample being examined by comparison with the *reference solution.* Each ml of the potassium nitrate *reference solution* is equivalent to 0.2000 mg of $C_3H_5N_3O_9$.

Disintegration test. Complies with the test for 5.3 Disintegration test for tablets and capsules. Time period: 2 minutes.

Uniformity of content. For the *sample solution,* place 10 powdered tablets in 10 separate centrifuge tubes containing a few glass beads, add 4.5 ml of glacial acetic acid R and 0.5 ml of water, shake for 1 hour, and centrifuge.

For the *reference solution,* dissolve 133.5 mg of potassium nitrate R, previously dried at 105 °C, in sufficient water to produce 50 ml. Transfer 10 ml to a volumetric flask and add sufficient glacial acetic acid R to produce 100 ml.

For tablets containing *0.4 to 0.6 mg of Glyceryl trinitrate,* use 1 ml of the clear *sample solution* and 1 ml of a mixture of equal volumes of the *reference solution* and glacial acetic acid R. To both solutions add 2 ml of phenoldisulfonic acid TS, mix, and allow to stand for 15 minutes. Then add 8 ml of water, mix well, allow to cool, and add slowly with swirling 10 ml of ammonia (~260 g/l) TS. Cool and dilute to 20 ml with water.

For tablets containing *0.2 to 0.3 mg of Glyceryl trinitrate,* use 2 ml of the clear *sample solution* and 2 ml of a mixture of 3 volumes of glacial acetic acid R and 1 volume of the *reference solution.* To both solutions add 2 ml of phenoldisulfonic acid TS, mix, and allow to stand for 15 minutes. Then add 8 ml of water, mix

well, allow to cool, and add slowly with swirling 10 ml of ammonia (~260 g/l) TS. Cool and dilute to 20 ml with water.

For tablets containing *less than 0.2 mg of Glyceryl trinitrate*, use 2 ml of the clear *sample solution* and 2 ml of a mixture of 7 volumes of glacial acetic acid R and 1 volume of the *reference solution*. To both solutions add 2 ml of phenoldisulfonic acid TS, mix, and allow to stand for 15 minutes. Then add 8 ml of water, mix well, allow to cool, and add slowly with swirling 10 ml of ammonia (~260 g/l) TS. Cool and dilute to 20 ml with water.

Measure the absorbances in the *sample* and *reference solutions* of all three concentrations at the maximum at about 405 nm against a solvent cell containing a reagent blank. Calculate the amount in mg of $C_3H_5N_3O_9$ in the sample being examined by comparison with the *reference solution*. Each ml of the potassium nitrate *reference solution* is equivalent to 0.2000 mg of $C_3H_5N_3O_9$.

The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

GRISEOFULVINI COMPRESSI
GRISEOFULVIN TABLETS

Category. Antifungal drug.

Labelling. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 125 mg, 250 mg.

Requirements

Comply with the monograph for "Tablets".

Griseofulvin tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_{17}H_{17}ClO_6$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Shake a quantity of the powdered tablets equivalent to 0.125 g of Griseofulvin with 20 ml of chloroform R and 1 g of anhydrous sodium sulfate R, and filter. Evaporate the filtrate to dryness and dry under reduced pressure

(not exceeding 0.7 kPa) for 1 hour. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from griseofulvin RS or with the *reference spectrum* of griseofulvin.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 1 volume of ethylmethylketone R and 1 volume of xylene R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 5 mg of Griseofulvin with 10 ml of chloroform R, filter, and use the clear filtrate. For solution (B) dissolve 5 mg of griseofulvin RS in 10 ml of chloroform R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powdered tablets equivalent to 5 mg of Griseofulvin add 1 ml of sulfuric acid (~1760 g/l) TS; a yellow-orange colour is produced. Add 1 drop of potassium dichromate (100 g/l) TS; the colour of the solution changes to wine-red.

Loss on drying. Dry a quantity of the powdered tablets equivalent to 0.1 g of Griseofulvin at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.08 g of Griseofulvin, accurately weighed, add 150 ml of ethyl acetate R and boil under a reflux condenser for 15 minutes. Cool, add sufficient dehydrated ethanol R to produce 200 ml, shake, and centrifuge. Dilute 2.0 ml of the supernatant liquid to 100 ml with dehydrated ethanol R.

Measure the absorbance of this solution in a 1-cm layer at the maximum of about 291 nm and calculate the content of $C_{17}H_{17}ClO_6$ using the absorptivity value of 68.6 ($A^{1\%}_{1\,cm}$ = 686).

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

IBUPROFENI COMPRESSI
IBUPROFEN TABLETS

Category. Non-steroidal anti-inflammatory drug.

Additional information. Strength in the current WHO Model list of essential medicines: 200 mg, 400 mg.

Requirements

Comply with the monograph for "Tablets".

Ibuprofen tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{13}H_{18}O_2$ stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.

To a quantity of the powdered tablets equivalent to about 0.8 g of Ibuprofen add 20 ml of acetone R, filter, and allow the filtrate to evaporate without heating. To the residue add 10 ml of acetone R, allow to crystallize, separate the crystals, dry in air, and use the dried crystals for the following tests.

A. Carry out the examination with the dried crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ibuprofen RS or with the *reference spectrum* of ibuprofen.

B. Dissolve 25 mg of the dried crystals in sufficient sodium hydroxide (0.1 mol/l) VS to produce 100 ml. The absorption spectrum of the resulting solution, when observed between 230 nm and 350 nm, exhibits maxima at about 265 nm and 273 nm, minima at about 245 nm and 271 nm, and a shoulder at about 259 nm.

C. Melting temperature of the dried crystals, about 76 °C.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R3 as the coating substance and a mixture of 15 volumes of hexane R, 5 volumes of ethyl acetate R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of the following 3 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.2 g of Ibuprofen with three 10-ml quantities of chloroform R, filter, evaporate the combined filtrates to a volume of about 1 ml, and add sufficient chloroform R to produce 2 ml. For solution (B) dilute 1

volume of solution A to 100 volumes with chloroform R. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray very lightly with a solution of 10 mg of potassium permanganate R per ml of sulfuric acid (~100 g/l) TS. Heat again at 120 °C for 20 minutes and examine the chromatogram in ultraviolet light (365 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.5 g of Ibuprofen add 60 ml of chloroform R and shake for 15 minutes. Filter through a fine glass microfibre paper (e.g. Whatman GF/F) under reduced pressure. Wash the residue with 2 quantities, each of 20 ml of chloroform R, and evaporate the combined filtrates in a current of air until just dry. Dissolve the residue in 100 ml of neutralized ethanol TS and titrate with sodium hydroxide (0.1 mol/l) VS, determining the end-point potentiometrically.

Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 20.63 mg of $C_{13}H_{18}O_2$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

INDOMETACINI COMPRESSI
INDOMETACIN TABLETS

Category. Non-steroidal anti-inflammatory drug.

Requirements

Comply with the monograph for "Tablets".

Indometacin tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{16}ClNO_4$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.1 g of Indometacin add 5 ml of chloroform R and shake. Filter and evaporate the filtrate to dryness. Dry the residue at 70 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury). Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region.

The infrared absorption spectrum is concordant with the spectrum obtained from indometacin RS or with the *reference spectrum* of indometacin.

B. To a quantity of the powdered tablets equivalent to about 0.05 g of Indometacin add 60 ml of ethanol (~750 g/l) TS and shake. Allow to stand for 10 minutes, shake again, and dilute with sufficient ethanol (~750 g/l) TS to produce 100 ml. Filter, discard the first 10 ml of filtrate, then dilute 5 ml of the filtrate to 100 ml with the same solvent. The absorption spectrum of the resulting solution, when observed between 300 nm and 350 nm, exhibits a maximum at about 318 nm.

C. To a quantity of the powdered tablets equivalent to about 25 mg add 10 ml of water, 2 drops of sodium hydroxide (~200 g/l) TS, shake, and filter. To the filtrate add 1.0 ml of sodium nitrite (10 g/l) TS, allow to stand for 5 minutes, and carefully add about 0.5 ml of hydrochloric acid (~250 g/l) TS; a green colour is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and preparing a slurry in sodium dihydrogen phosphate (45 g/l) TS. As the mobile phase, use a mixture of 7 volumes of ether R and 3 volumes of light petroleum R1. Apply separately to the plate 5 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.1 g of Indometacin with 5 ml of chloroform R, filter, and use the filtrate. For solution (B) dilute 1 volume of solution A to 20 volumes with chloroform R, further dilute 1 volume of this solution to 10 volumes with the same solvent. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent of about 0.05 g of Indometacin add 10 ml of water and allow to stand for 15 minutes, swirling occasionally. Add 75 ml of methanol R, shake well, add sufficient methanol R to produce 100 ml, and filter. To 5 ml of the filtrate add a mixture of equal volumes of methanol R and phosphate buffer pH 7.2, TS to produce 100 ml. Measure the absorbance of a 1-cm layer at the maximum at about 318 nm against a solvent cell containing the above solvent mixture.

Calculate the percentage content of $C_{19}H_{16}ClNO_4$ using the absorptivity value of 19.3 ($A^{1\%}_{1\,cm} = 193$).

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

ISONIAZIDI COMPRESSI
ISONIAZID TABLETS

Category. Antituberculosis drug.

Additional information. Strength in the current WHO Model list of essential medicines: 100–300 mg.

Requirements

Comply with the monograph for "Tablets".

Isoniazid tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_6H_7N_3O$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.1 g of Isoniazid add 10 ml of ethanol (~750 g/l) TS and shake for 15 minutes. Centrifuge and decant the supernatant liquid. Extract the remaining liquid with two further 10-ml quantities of ethanol (~750 g/l) TS and evaporate the combined extracts to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS or with the *reference spectrum* of isoniazid.

B. To a quantity of the powdered tablets equivalent to about 0.1 g of Isoniazid add 2.0 ml of water, shake, and filter. Then add a mixture composed of 1.0 ml of silver nitrate (40 g/l) TS and 1.0 ml of ammonia (~100 g/l) TS; bubbles of nitrogen evolve, the mixture turns from yellow to black and a metallic silver mirror appears on the sides of the test-tube.

C. To a quantity of the powdered tablets equivalent to about 1 mg of Isoniazid add 50 ml of ethanol (~750 g/l) TS, shake, and filter. To 5 ml of the filtrate add 0.1 g of sodium tetraborate R and 5 ml of 1-chloro-2,4 dinitrobenzene/ethanol TS, evaporate to dryness on a water-bath, and continue heating for a further 10 minutes. To the residue add 10 ml of methanol R and mix; a reddish violet colour is produced.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 5 volumes of ethyl acetate R, 2 volumes of acetone R, 2 volumes of methanol R, and 1 volume of water as the mobile phase. Apply separately to the plate

10 µl of each of the 3 following solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.1 g of Isoniazid with 10 ml of methanol R, filter, and use the filtrate. For solution (B) use 10 mg of isoniazid RS per ml of methanol R. For solution (C) dilute 1 volume of solution A to 100 volumes with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets equivalent to about 0.4 g of Isoniazid as completely as possible in water, filter, and wash the residue with sufficient water to produce 250 ml. Place 50 ml of the resulting solution in a titration vessel, add 50 ml of water, 20 ml of hydrochloric acid (~250 g/l) TS, and 0.2 g of potassium bromide R, and titrate with potassium bromate (0.0167 mol/l) VS as described under 2.7 Nitrite titration.

Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 3.429 mg of $C_6H_7N_3O$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

MEBENDAZOLI COMPRESSI
MEBENDAZOLE TABLETS

Category. Anthelmintic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg.

Requirements

Comply with the monograph for "Tablets".

Mebendazole tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{13}N_3O_3$ stated on the label.

Identity tests

A. See the test described below under "Related substances". The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution D.

B. Shake a quantity of the powdered tablets equivalent to 0.04 g of Mebendazole with 2 ml of sodium hydroxide (~80 g/l) TS and heat the yellowish coloured suspension until dissolved; the solution is yellow. Add a few drops of copper (II) sulfate (160 g/l) TS; a greenish precipitate is produced. Add a few drops of ammonia (~100 g/l) TS; the colour of the precipitate turns to greenish blue.

C. To a quantity of the powdered tablets equivalent to 0.04 g of Mebendazole add 2 ml of sulfuric acid (~1760 g/l) TS; a yellow solution is produced. Carefully dilute with 3 ml of water; the yellow colour disappears. Filter and add 1 ml of silver nitrate (40 g/l) TS; a white precipitate is formed which does not dissolve in an excess of ammonia (~100 g/l) TS.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 90 volumes of chloroform R, 5 volumes of methanol R, and 5 volumes of anhydrous formic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following four solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 50 mg of Mebendazole with a mixture of 1 ml of anhydrous formic acid R and 9 ml of chloroform R, filter, and use the clear filtrate. For solution (B) dilute 5 ml of solution A to 10 ml using the same mixture of solvents, and for solution (C) dilute 0.5 ml of solution A to 10 ml using the same mixture of solvents. For solution (D) dissolve 12.5 mg of mebendazole RS in 5 ml of the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.1 g of Mebendazole, accurately weighed, to a 100-ml volumetric flask with 50 ml of anhydrous formic acid R. Heat in a water-bath at 50 °C for 15 minutes. Cool, add water to volume, mix, and filter through a sintered-glass filter. Transfer 10 ml of the filtrate to a 250-ml separator, add 50 ml of water and 50 ml of chloroform R. Shake for about 2 minutes, allow the phases to separate, and transfer the chloroform layer to a second 250-ml separator. Wash the aqueous layer with two portions, each of 10 ml, of chloroform R, adding the washings to the second separator, and discard the aqueous layer. Wash the combined chloroform extracts with a mixture of 4 ml of hydrochlo-

ric acid (0.1 mol/l) VS and 50 ml of a previously diluted solution of 5 ml of anhydrous formic acid R with 45 ml of water. Transfer the chloroform layer to a 100-ml volumetric flask. Extract the aqueous washings with two portions, each of 10 ml, of chloroform R, add these chloroform extracts to the chloroform solution in the volumetric flask, dilute with 2-propanol R to volume, and mix. Further dilute 5 ml of this solution to 100 ml with 2-propanol R, and mix. For the *reference solution* transfer 20 mg of mebendazole RS, accurately weighed, to a 100-ml volumetric flask and add 90 ml of chloroform R, 7 ml of 2-propanol R, and 2 ml of a mixture of 0.2 ml of anhydrous formic acid R and 1.8 ml of water. Shake until the solid has dissolved, add 2-propanol R to volume, and mix. Transfer 5 ml of this solution to a 200-ml volumetric flask, dilute with 2-propanol R to volume, and mix. For the reagent blank mix 45 ml of chloroform R with 1 ml of a mixture of 0.1 ml of anhydrous formic acid R and 0.9 ml of water using a 100-ml volumetric flask, dilute to volume with 2-propanol R, and mix. Transfer 5 ml of this solution to a second 100-ml volumetric flask, dilute to volume with 2-propanol R, and mix.

Without delay measure the absorbance of the *sample* and the *reference solutions* in a 1-cm layer at the maximum at about 247 nm against a solvent cell containing the reagent blank. Calculate the amount in mg of $C_{16}H_{13}N_3O_3$ in the sample being examined using the following formula: $20C(A_u/A_s)$, in which C is the concentration, in mg per ml, of mebendazole RS in the *reference solution,* and A_u and A_s are the absorbances for the *sample* and *reference solutions,* respectively.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

MELARSOPROLI INJECTIO
MELARSOPROL INJECTION

Description. Melarsoprol injection is a clear, colourless to almost colourless solution.

Category. Antitrypanosomal drug.

Storage. Melarsoprol injection should be protected from light.

Additional information. Strength in the current WHO Model list of essential medicines: 3.6%.

Melarsoprol (formula: $C_{12}H_{15}AsN_2OS_2$) has a relative molecular mass of 398.3 and the chemical name: 2-[4-(4,6-Diamino-1,3,5-triazin-2-ylamino)phenyl]-1,3,2-dithiarsolan-4-yl-methanol; CAS Reg. No. 494-79-1.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Melarsoprol injection is a sterile solution of melarsoprol in propylene glycol, containing 5% of water.

The injection is normally prepared by heating equimolar amounts of melarsen oxide and dimercaprol in propylene glycol and adding water. The solution is sterilized by "Heating in an autoclave" or by any other suitable method (see 5.8 Methods of sterilization).

Melarsoprol injection contains not less than **3.4%** and not more than **3.8%** of $C_{12}H_{15}AsN_6OS_2$.

Identity tests

A. To a volume of the injection equivalent to 35 mg of Melarsoprol, add 5 ml of dilute hypophosphorous acid TS and heat on a water-bath; a yellow precipitate is produced.

B. To a similar volume of the injection as for test A add 4 ml of sodium hydroxide (~300 g/l) TS and heat to boiling; the evolved vapours turn red litmus paper R to blue.

C. To a further volume of the injection as for test A add 2 ml of water, 1 ml of ammonia (~260 g/l) TS, and 3 ml of silver nitrate (40 g/l) TS; a yellow precipitate is produced which is soluble in nitric acid (~1000 g/l) TS.

Relative density. $d_{20}^{20} = 1.050–1.056$.

Inorganic arsenic. To a volume of the injection equivalent to 0.18 g of Melarsoprol add 4 ml of sodium hydroxide (1 mol/l) VS, shake, and add 3 ml of sulfuric acid (~190 g/l) TS; a precipitate is produced. Shake, dilute to 50 ml with water, and mix. Allow to stand for 2–3 minutes, filter, and dilute 10 ml of the clear filtrate to 100 ml with water. Use 30 ml of this solution and proceed as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 30 mg/g.

Clarity and colour of solution. The injection is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under 1.11 Colour of liquids.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.5 g of the injection; the water content is not less than 40 mg/g and not more than 60 mg/g.

Assay. Transfer an accurately measured volume of the injection equivalent to about 0.18 g of Melarsoprol to a long-necked, 200-ml flask. While cooling, add a mixture of 12 ml of sulfuric acid (~1760 g/l) TS and 40 ml of hydrogen peroxide (~330 g/l) TS. Heat gently and carefully until white vapours appear in the neck of the flask (heating time: 45–60 minutes). Cool slightly, add 8 g of potassium sulfate R and 0.05 g of starch R, and heat to boiling until decolorization occurs (heating time: 60–90 minutes). Cool, and add slowly 70 ml of water. Transfer to a conical flask, rinse with 50 ml of water, add 0.05 ml of phenolphthalein/ethanol TS, then add slowly while cooling 30–40 ml of sodium hydroxide (~300 g/l) TS until a light pink colour is obtained. Add, drop by drop, sulfuric acid (~190 g/l) TS until decolorized, then add 3 g of sodium hydrogen carbonate R and 50 ml of water. Titrate with iodine (0.05 mol/l) VS.

Each ml of iodine (0.05 mol/l) VS is equivalent to 19.92 mg of $C_{12}H_{15}AsN_6OS_2$.

METRONIDAZOLI INJECTIO
METRONIDAZOLE INJECTION

Description. Metronidazole injection is a colourless solution.

Category. Antibacterial drug; antiamoebic drug.

Storage. Metronidazole should be kept in a single-dose container, protected from light.

Additional information. Strength in the current WHO Model list of essential medicines: 5 mg/ml.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Metronidazole injection is a sterile solution of metronidazole in water for injections.

The solution is sterilized by a suitable method (see 5.8 Methods of sterilization).

Metronidazole injection contains not less than **90.0%** and not more than **110.0%** of the amount of $C_6H_9N_3O_3$ stated on the label.

Identity tests

A. Dilute a volume of the injection equivalent to 20 mg of Metronidazole to 100 ml with a mixture of solvents composed of 1 ml of sulfuric acid (~1760 g/l) TS in 350 ml of methanol R. Further dilute 1 ml of this solution to 10 ml using the same mixture of solvents. The absorption spectrum, when observed between 220 nm and 350 nm, is qualitatively similar to that of a 20 µg/ml solution of metronidazole RS in the same mixture of solvents.

B. To a volume of the injection equivalent to 5 mg of Metronidazole, add 0.05 g of 4-dimethylaminobenzaldehyde R dissolved in 2 ml of hydrochloric acid (~70 g/l) TS; a yellowish colour is produced. Add 0.05 g of zinc R powder; the colour changes to red-orange.

pH value. pH of the injection, 4.5–7.0.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and acetone R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) evaporate a volume of the injection equivalent to 0.05 g of Metronidazole to dryness on a water-bath and dissolve the residue in 5 ml of a mixture of equal volumes of methanol R and chloroform R. For solution (B) dissolve 20 mg of 2-methyl-5-nitroimidazole R in 10 ml of the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The spot obtained with solution B is more intense than any corresponding spot obtained with solution A.

Assay. Transfer an accurately measured volume of the solution equivalent to about 10 mg of Metronidazole to a 100-ml volumetric flask. Add 80 ml of hydrochloric acid (0.1 mol/l) VS and shake. Dilute to volume with the same acid and mix well. Filter through a dry filter-paper, discarding the first few ml of filtrate. Dilute accurately 5.0 ml of the filtrate to 200 ml with hydrochloric acid (0.1 mol/l) VS and mix well.

Measure the absorbance of the resulting solution in a 1-cm layer at the maximum at about 277 nm. Calculate the content of $C_6H_9N_3O_3$, using the absorptivity value of 37.7 ($A^{1\%}_{1\,cm} = 377$).

Bacterial endotoxins. Carry out the test as described under "3.4 Test for bacterial endotoxins". Dilute the injection, if necessary, with water LAL to give a solution containing 5 mg per ml (solution A). Solution A contains not more than 3.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

METRONIDAZOLI COMPRESSI
METRONIDAZOLE TABLETS

Category. Anti-amoebic drug; antibacterial drug.

Additional information. Strength in the current WHO Model list of essential medicines: 200–500 mg.

Requirements

Comply with the monograph for "Tablets".

Definition. Metronidazole tablets may be film-coated and not necessarily circular in shape.

Metronidazole tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_6H_9N_3O_3$ stated on the label.

Identity tests
To a quantity of the powdered tablets equivalent to 60 mg of Metronidazole add 20 ml of water, shake, and filter. Evaporate the filtrate to a smaller volume, allow to crystallize, separate the crystals, dry at 105 °C for 1 hour, and use the dried material for tests A and B.

A. Dissolve 20 mg of the dried material in 100 ml of a mixture of solvents composed of 1 ml of sulfuric acid (~1760 g/l) TS in 350 ml of methanol R. Further dilute 1 ml of this solution to 10 ml using the same mixture of solvents. The absorption spectrum, when observed between 220 nm and 350 nm, is qualitatively similar to that of a 20 µg/ml solution of metronidazole RS in the same mixture of solvents.

B. To 25 mg of the dried material add 0.05 g of 4-dimethylaminobenzaldehyde R dissolved in 2 ml of hydrochloric acid (~70 g/l) TS; a yellowish colour is produced. Add 0.05 g of zinc R powder; the colour changes to red-orange.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and acetone R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.2 g of Metronidazole with 5 ml of a mixture of equal volumes of methanol R and chloroform R for 5 minutes, filter, and use the clear filtrate. For solution (B) dissolve 20 mg of 2-methyl-5-nitroimidazole R in 10 ml of the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The spot obtained with solution B is more intense than any corresponding spot obtained with solution A.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder, equivalent to about 0.2 g of Metronidazole, accurately weighed, to a sintered-glass filtering crucible, and extract with 6 quantities, each of 10 ml, of hot acetone R. Cool, add to the combined extracts 50 ml of acetic anhydride R and 0.1 ml of brilliant green/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Repeat the procedure without the powdered tablets being examined and make any necessary corrections.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 17.12 mg of $C_6H_9N_3O_3$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

MORPHINI SULFATIS COMPRESSI
MORPHINE SULFATE TABLETS

Category. Opioid analgesic.

Additional information. Strength in the current WHO Model list of essential medicines: 10 mg.

Requirements

Comply with the monograph for "Tablets".

Morphine sulfate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.1 g of Morphine sulfate add 10 ml of ethanol (~750 g/l) TS and shake for 15 minutes. Centrifuge and decant the supernatant liquid. Extract the remaining liquid with two further 10-ml quantities of ethanol (~750 g/l) TS and evaporate the combined extracts to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of morphine sulfate.

B. To a quantity of the powdered tablets equivalent to about 20 mg of Morphine sulfate add 5 ml of water, shake, and filter. To the filtrate add 0.05 ml of ferric chloride (25 g/l) TS; a blue colour is produced.

C. To a quantity of the powdered tablets equivalent to about 20 mg of Morphine sulfate add 5 ml of sulfuric acid (0.05 mol/l) VS, shake, and filter. To the filtrate add 0.5 ml of a saturated solution of potassium iodate R; an amber colour is produced which reaches maximum intensity after 5 minutes. Add 0.5 ml of ammonia (~260 g/l) TS; the colour darkens almost to black.

D. To a quantity of the powdered tablets equivalent to about 20 mg of Morphine sulfate add 5 ml of water, shake, and filter. The filtrate yields the reactions described under 2.1 General identification tests as characteristic of sulfates.

Assay. Weigh and powder 20 tablets. To a quantity of the powdered tablets equivalent to about 0.4 g of Morphine sulfate add 25 ml of water, 5 ml of sodium hydroxide (1 mol/l) VS, and 1 g of ammonium sulfate R, swirl to dissolve. Add 20 ml of ethanol (~750 g/l) TS and extract with successive quantities of 40 ml, 20 ml, 20 ml, and 20 ml of a mixture of 3 volumes of chloroform R and 1 volume of ethanol (~750 g/l) TS. Wash each extract with the same 5 ml of water, filter, and evaporate the solvent. Dissolve the residue in 10 ml of hydrochloric acid (0.05 mol/l) VS, boil, cool, add 15 ml of water and a few drops of methyl red/ethanol TS. Titrate the excess acid with sodium hydroxide (0.05 mol/l) VS.

Each ml of hydrochloric acid (0.05 mol/l) VS is equivalent to 18.97 mg of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

NICLOSAMIDI COMPRESSI
NICLOSAMIDE TABLETS

Category. Anthelmintic drug.

Labelling. The designation on the container should state that the tablets should be chewed thoroughly before being swallowed.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Chewable Niclosamide tablets may contain sweetening and flavouring agents.

Requirements

Comply with the monograph for "Tablets".

Niclosamide tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_{13}H_8Cl_2N_2O_4$ stated on the label.

Identity tests

Heat a quantity of the powdered tablets equivalent to 0.5 g of Niclosamide with 25 ml of ethanol (~750 g/l) TS, filter while hot, and evaporate to dryness on a water-bath.

- The residue complies either with test A alone or with tests B, C, and D.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from niclosamide RS or with the *reference spectrum* of niclosamide.

B. Using a small flame heat 0.05 g of the residue in a test-tube. Collect and dissolve the sublimate in 5 ml of water and add a few drops of ferric chloride (25 g/l) TS; a violet colour is produced.

C. To 0.05 g of the residue add 5 ml of hydrochloric acid (1 mol/l) VS and 0.1 g of zinc R powder and heat in a water-bath for 10 minutes. Cool and filter. To the filtrate add 0.5 ml of sodium nitrite (10 g/l) TS and allow to stand for 10 minutes. Add 2 ml of ammonium sulfamate (25 g/l) TS, shake, allow to stand for 10 minutes, and add 2 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS; a deep red colour is produced.

D. Dissolve 0.1 g of the residue in 1 ml of acetic anhydride R and boil for 10 minutes. Cool and add 10 ml of water. Collect the precipitate on a filter, wash with water, recrystallize from ethanol (~750 g/l) TS, and dry at 105 °C; melting temperature of the acetyl derivative, about 178 °C.

2-Chloro-4-nitroaniline. To a quantity of the powdered tablets equivalent to 0.1 g of Niclosamide add 20 ml of methanol R and boil for 2 minutes. Cool, add sufficient hydrochloric acid (1 mol/l) VS to produce 50 ml, and filter. To 10 ml of the filtrate add 1 ml of sodium nitrite (1 g/l) TS and allow to stand for 10 minutes. Add 1 ml of ammonium sulfamate (25 g/l) TS, shake, allow to stand for 10 minutes, and add 1 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS; the colour produced is not more intense than that produced by a similarly and simultaneously treated solution containing 10 µg of 2-chloro-4-nitroaniline R.

5-Chlorosalicylic acid. To a quantity of the powdered tablets equivalent to 0.5 g of Niclosamide add 10 ml of water and boil. Cool, filter, and add to the filtrate a few drops of ferric chloride (25 g/l) TS; no red or violet colour is produced.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.3 g of Niclosamide, accurately weighed, add 60 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS, determining the endpoint potentiometrically as described under 2.6 Non-aqueous titration, Method B.

Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 32.71 mg of $C_{13}H_8Cl_2N_2O_4$.

NITROFURANTOINI COMPRESSI
NITROFURANTOIN TABLETS

Category. Antibacterial drug.

Additional information. Strength in the current, WHO Model list of essential medicines: 100 mg.

Nitrofurantoin tablets may have an enteric sugar coating.

Requirements

Comply with the monograph for "Tablets".

Nitrofurantoin tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_8H_6N_4O_5$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. To a quantity of the powdered tablets equivalent to 0.1 g of Nitrofurantoin add 10 ml of acetic acid (~300 g/l) TS, boil for a few minutes, and filter while hot. Cool to room temperature, collect the precipitate, and dry at 105 °C for 1 hour. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from nitrofurantoin RS or with the *reference spectrum* of nitrofurantoin.

B. Carry out the test in subdued light.

The absorption spectrum of the final solution obtained in the "Assay", when observed between 220 nm and 400 nm, exhibits two maxima at about 266 nm and 367 nm. The ratio of the absorbance at 367 nm to that at 266 nm is between 1.36 and 1.42.

C. To a quantity of the powdered tablets equivalent to 25 mg of Nitrofurantoin add a mixture of 25 ml of water and 1 ml of ammonia (~260 g/l) TS, shake, and filter; a yellow-orange colour is produced. Add 5 ml of silver nitrate (40 g/l) TS; a yellow precipitate is produced (distinction from furazolidone and nitrofural).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 9 volumes of nitromethane R and 1 volume of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.1 g of Nitrofurantoin with 10 ml of a mixture of 1 volume of dimethylformamide R and 9 volumes of acetone R, filter, and use the clear filtrate. For solution (B) dilute 1 ml of solution A to 100 ml with acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air, heat it at 105 °C for 5 minutes, and examine the chromatogram in ultraviolet light (254 nm). Spray the warm plate with phenylhydrazine/hydrochloric acid TS, heat it again at 105 °C for 10 minutes, and examine the chromatogram in daylight.

By either method of visualization any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

The operations described below must be carried out in subdued light.

Weigh and powder 20 tablets. Add a quantity of the powder equivalent to about 0.12 g of Nitrofurantoin, accurately weighed, to 50 ml of dimethylformamide R, shake for 5 minutes, and add sufficient water to produce 1000 ml. Mix and dilute 5 ml to 100 ml with an aqueous solution containing 1.8 g of sodium acetate R and 0.14 ml of glacial acetic acid R in 100 ml, and filter.

Measure the absorbance of the filtrate in a 1-cm layer at the maximum at about 367 nm, using as the blank the mixture as prepared above of sodium acetate and glacial acetic acid. Calculate the content of $C_8H_6N_4O_5$, using the absorptivity value of 76.5 ($A^{1\%}_{1\,cm} = 765$).

NYSTATINI COMPRESSI
NYSTATIN TABLETS

Category. Antifungal drug.

Storage. Nystatin tablets should be kept in a tightly closed container.

Labelling. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 500 000 IU.

Nystatin tablets may be coated.

Requirements

Comply with the monograph for "Tablets".

Identity tests

A. To a quantity of the powdered tablets equivalent to 0.1 g of Nystatin add a mixture of 5 ml of glacial acetic acid R and 50 ml of methanol R, shake, add sufficient methanol R to produce 100 ml, and filter. Dilute 1 ml of the filtrate to 100 ml with methanol R. The absorption spectrum of the resulting solution, when observed between 240 nm and 350 nm, exhibits 3 maxima at about 291 nm, 305 nm, and 319 nm; the ratio of the absorbance of a 1-cm layer at 291 nm to that at 305 nm is between 0.61 and 0.73, and the ratio of the absorbance at 319 nm to that at 305 nm is between 0.83 and 0.96.

B. To a quantity of the powdered tablets equivalent to 0.05 g of Nystatin add 2 ml of sulfuric acid (~1760 g/l) TS; a brown-violet colour is produced.

C. To a quantity of the powdered tablets equivalent to 0.05 g of Nystatin add 2 ml of ethanol (~750 g/l) TS, shake, and filter. To the filtrate add 1 ml of hydrochloric acid (~250 g/l) TS, and 2 drops of a solution composed of 1 ml of ferric chloride (25 g/l) TS and 10 ml of water; the yellow colour of the solution becomes more intense.

Loss on drying. Dry a quantity of the powdered tablets equivalent to 0.1 g of Nystatin at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours; it loses not more than 50 mg/g.

Assay

The operations described below must be carried out in subdued light.

Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 200 000 IU of Nystatin, accurately weighed, add 50 ml of dimethylformamide R and shake for 1 hour. Centrifuge, and dilute 10 ml of the clear, supernatant liquid to 200 ml with sterile phosphate buffer pH 6.0, TS3. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using Petri dishes or rectangular trays filled to a depth of 1–2 mm with culture medium Cm3 having a final pH of 6.0–6.2 and inoculated with *Saccharomyces cerevisiae* (NCYC 87; ATCC 9763) as the test organism, adding an appropriate concentration of Nystatin (usually between 25 and 300 IU per ml) and incubating at a temperature of 29–33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105%.

The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the activity prescribed or stated on the label and expressed in International Units.

Disintegration. Carry out 5.3 Disintegration test for tablets and capsules, but using hydrochloric acid (0.1 mol/l) VS instead of water, and operate the apparatus for 30 minutes. If any tablets have not disintegrated, wash them rapidly by immersion in water and continue the test using phosphate standard buffer, pH 6.8, TS; all tablets must have disintegrated within a further 30 minutes.

SALES PERORALES AD REHYDRATATIONEM
ORAL REHYDRATION SALTS

Definition. Oral Rehydration Salts (ORS) are dry mixtures of powders containing per packet:

sodium chloride	NaCl	2.6 g
trisodium citrate dihydrate	$C_6H_5Na_3O_7,2H_2O$	2.9 g
potassium chloride	KCl	1.5 g
anhydrous glucose	$C_6H_{12}O_6$	13.5 g

Before administration the contents of each packet should be dissolved in 1 litre of water.

Description. A white, crystalline powder; odourless.

Category. Used for the prevention and treatment of dehydration due to diarrhoea, including maintenance therapy.

Storage. Oral Rehydration Salts should be kept in a sealed packet; if a free-flowing powder is required, it should be kept in an airtight packet, preferably made of aluminium laminate.

Labelling. The designation on the packet of Oral Rehydration Salts should state: (1) the total net mass and the mass of the contents of each constituent, both expressed in grams, (2) the required volume of water to reconstitute the solution, (3) directions for the preparation of the solution and its administration, and (4) a warning that any solution that remains unused 24 hours after preparation is to be discarded.

Additional information. In the formulation of Oral Rehydration Salts trisodium citrate dihydrate may be replaced by 2.5 g/l of sodium hydrogen carbonate, $NaHCO_3$ (sodium bicarbonate). However, as the stability of the latter formulation under tropical conditions is very poor, it is recommended only in Oral Rehydration Salts manufactured for immediate use, or where sodium hydrogen carbonate is packaged in separate packets. These formulations would also allow the use of 14.85 g/l of glucose monohydrate, $C_6H_{12}O_6,H_2O$, instead of anhydrous glucose.

The title of the two formulations could be distinguished by: "ORS-citrate" or "OSR-hydrogen carbonate" (bicarbonate). The title Oral Rehydration Salts (ORS) used without qualification implies that the product is the citrate formulation as defined above.

Oral Rehydration Salts may contain suitable pharmaceutical aids, in minimal quantities, to improve the flow characteristics and/or the flavour.

Requirements

These specifications apply only to ORS-citrate.

One to three single doses may represent a complete treatment; therefore, the contents of each packet should comply with the following requirements.

Oral Rehydration Salts contain not less than **90.0%** and not more than **110.0%** of the equivalent amounts of sodium Na^+, potassium K^+, chlorides Cl^-, citrate $C_6H_5O_7^{3-}$ of the relevant constituents stated on the label, and not less than **90.0%** and not more than **110.0%** of the amount of anhydrous glucose $C_6H_{12}O_6$ stated on the label.

Identity tests

A. Melts when heated; first becomes yellow then brown, swells up and burns, evolving an odour of burnt sugar.

Dissolve the entire contents of one packet in 250 ml of water to prepare the test solution to be used in tests B, C, D, E, and F.

B. The test solution yields reaction A described under 2.1 General identification tests as characteristic of sodium.

C. To 5 ml of the test solution add 4 drops of sodium cobaltinitrite (100 g/l) TS; a yellow-orange precipitate is produced (potassium).

D. A 5-ml aliquot of the test solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

E. A 5-ml aliquot of the test solution after neutralization yields reaction A described under 2.1 General identification tests as characteristic of citrates.

F. Add a few drops of the test solution to 5 ml of hot potassio-cupric tartrate TS; a copious red precipitate is produced (glucose).

Uniformity of mass. Weigh the contents of 20 packets selected at random and determine the average mass. Not more than two of the individual masses deviate from the average mass by more than 5% and none deviates by more than 10%.

Loss on drying. Dry to constant mass at 50 °C; it loses not more than 20 mg/g.

pH value. pH of the solution reconstituted as directed on the label, 7.0–8.8.

Assays

Carry out all the assays on quantities taken from a single packet. If the quantity of one packet is insufficient to carry out all the assays, take another packet for the assay for citrates and for the assay for glucose from the same batch.

Prepare the following solution (= *solution A*) for the assays for sodium, potassium, and chlorides. Dissolve about 8 g of ORS, accurately weighed, in sufficient water to produce 500 ml.

Sodium. Dilute 3 ml of solution A to 500 ml with water and determine by flame photometry as described under 1.8 Atomic spectrometry: emission and absorption at a wavelength of 589 nm. For the preparation of the reference solutions, use a stock standard solution prepared by dissolving sodium chloride R, previously dried to constant mass at 130 °C, in 1000 ml of water to contain 508.4 mg of NaCl (0.2 mg of Na^+ per ml).

Each g of sodium chloride and of trisodium citrate dihydrate is equivalent to 0.3934 g and 0.2345 g of Na^+, respectively.

Potassium. Dilute 3 ml of solution A to 500 ml with water and determine by flame photometry as described under 1.8 Atomic spectrometry: emission and absorption at a wavelength of 767 nm. For the preparation of the reference solutions, use a stock standard solution prepared by dissolving potassium chloride R, previously dried to constant mass at 130 °C, in 1000 ml of water to contain 190.6 mg of KCl (0.1 mg of K^+ per ml).

Each g of potassium chloride is equivalent to 0.5245 g of K^+.

Chlorides. Titrate 20 ml of solution A with silver nitrate (0.1 mol/l) VS, using potassium chromate (100 g/l) TS as indicator.

Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 3.545 mg of Cl^-.

Each g of sodium chloride and of potassium chloride is equivalent to 0.6066 g and 0.4756 g of Cl^-, respectively.

Citrates. Disperse about 2.8 g of ORS, accurately weighed, in 80 ml of glacial acetic acid R1, heat to about 50 °C, cool, dilute to 100 ml with glacial acetic acid R1, and allow to stand for 10 minutes. To 20 ml of the supernatant liquid add 0.25 ml of 1-naphtholbenzein/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 6.303 mg of $C_6H_5O_7^{3-}$. Each g of sodium citrate is equivalent to 0.6430 g of $C_6H_5O_7^{3-}$.

Glucose. Dissolve about 8.0 g of ORS, accurately weighed, in 40 ml of water, add 0.2 ml of ammonia (~100 g/l) TS, and dilute to 50 ml with water. Mix and allow to stand for 30 minutes. Determine the "Optical rotation" and calculate the quantity, in g, of anhydrous glucose $C_6H_{12}O_6$ by multiplying the observed rotation in degrees by 0.9477.

PARACETAMOLI COMPRESSI
PARACETAMOL TABLETS

Other name. Acetaminophen tablets.

Category. Non-opioid analgesic; antimigraine drug.

Storage. Paracetamol tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 100–500 mg.

Requirements

Comply with the monograph for "Tablets".

Definition. Paracetamol tablets may not necessarily be circular in shape.

Paracetamol tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_8H_9NO_2$ stated on the label.

Identity tests

To a quantity of the powdered tablets equivalent to 1 g of Paracetamol add 40 ml of acetone R, filter, evaporate the filtrate to dryness, and use the residue.

- The residue complies either with test A alone or with tests B and C. (Keep the remaining residue for "4-Aminophenol".)

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from paracetamol R or with the *reference spectrum* of paracetamol.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To 0.1 g of the residue add 2 ml of hydrochloric acid (~250 g/l) TS, and heat to boiling for 1 minute. Following this add 10 ml of water and 1 drop of potassium dichromate (100 g/l) TS, and shake; a violet colour slowly develops and does not become red.

4-Aminophenol. Dissolve 0.5 g of the residue in a mixture of equal volumes of methanol R and water and dilute to 10 ml with this mixture of solvents. Add 0.2 ml of alkaline sodium nitroprusside TS, mix, and allow to stand for 30 minutes. Prepare similarly a *reference solution* containing 0.5 g of 4-aminophenol-free paracetamol R and 0.5 ml of a solution containing 0.05 mg/ml of 4-aminophenol R in the same mixture of solvents. The colour of the *test solution* is not more intense than that of the *reference solution* (0.05 mg/g).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 65 volumes of chloroform R, 25 volumes of acetone R, and 10 volumes of

toluene R as the mobile phase, but allowing the solvent front to ascend 14 cm above the line of application in an unsaturated chamber.

Powder not less than 10 tablets and prepare the following six solutions. For solution (A) transfer a quantity of the powder equivalent to 200 mg of Paracetamol to a ground-glass-stoppered 15-ml centrifuge tube, add 10 ml of ethanol (~750 g/l) TS, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained, decant, and apply 40 µl. For solution (B) dissolve 20 mg of paracetamol RS per ml in ethanol (~750 g/l) TS and apply 40 µl. For solution (C) transfer a quantity of the powder equivalent to 1 g of Paracetamol to a ground-glass-stoppered 15-ml centrifuge tube, add 5 ml of peroxide-free ether R, shake mechanically for 30 minutes, centrifuge at 1000 revolutions per minute for 15 minutes or until a clear supernatant liquid is obtained, decant, and apply 200 µl. For solution (D) dilute 1 ml of solution C with sufficient ethanol (~750 g/l) TS to produce 10 ml and apply 40 µl. For solution (E) transfer 0.5 ml of solution B to a 10-ml volumetric flask, add 25 mg of 4′-chloroacetanilide R, dilute to volume with ethanol (~750 g/l) TS, and apply 40 µl. For solution (F) dissolve 10 mg of 4′-chloroacetanilide R in sufficient ethanol (~750 g/l) TS to produce 20 ml, further dilute 1 ml to 10 ml with the same solvent, and apply 40 µl. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and examine the chromatogram in ultraviolet light (254 nm).

The spot obtained with solution C, other than the principal spot, is not more intense than the corresponding spot (same R_f value) obtained with solution E. Any spot obtained with solution D, other than the principal spot, is not more intense than that obtained with solution E. The test is valid only if the chromatogram obtained with solution E shows two clearly separated spots.

Assay. Weigh and powder 20 tablets. Add a quantity of the powder equivalent to about 0.15 g of Paracetamol, accurately weighed, to 50 ml of sodium hydroxide (0.1 mol/l) VS, dilute with 100 ml of water, shake for 15 minutes, and add sufficient water to produce 200 ml. Mix, filter, and dilute 10 ml of the filtrate to 100 ml with water. Add 10 ml of this solution to 10 ml of sodium hydroxide (0.1 mol/l) VS and dilute to 100 ml with water.

Measure the absorbance of the resulting solution in a 1-cm layer at the maximum at about 257 nm and calculate the content of $C_8H_9NO_2$ using the absorptivity value of 71.5 ($A^{1\%}_{1\,cm} = 715$).

PENTAMIDINI ISETIONATIS PULVIS AD INJECTIONEM

PENTAMIDINE ISETIONATE POWDER FOR INJECTIONS

Description. A white or almost white, crystalline powder; odourless.

Category. Antileishmaniasis drug.

Labelling. The designation on the container should state that Pentamidine isetionate injection should be used immediately after preparation. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 200 mg.

Pentamidine isetionate is hygroscopic. The injection deteriorates on storage.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Pentamidine isetionate powder for injections is a sterile powder of pentamidine isetionate.

The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

The container of Pentamidine isetionate powder for injections contains not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$ stated on the label.

Identity tests

- Either test A alone or tests B and C may by applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pentamidine isetionate RS or with the *reference spectrum* of pentamidine isetionate.

B. The absorption spectrum of a solution containing a quantity of the powder for injections equivalent to 10 mg Pentamidine isetionate per ml of hydrochloric acid (0.01 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 262 nm; the absorbance of a 1-cm layer at this wavelength is about 0.47.

C. To a quantity of the powder for injections equivalent to 0.5 g of Pentamidine isetionate add 5 ml of water and heat to 80 °C to dissolve. Add 10 ml of sodium hydroxide (~50 g/l) TS, cool in ice, and filter. To 2 ml of the filtrate add 0.2 ml of nitric acid (~1000 g/l) TS followed by 0.2 ml of ceric ammonium nitrate TS; a red-orange colour is produced.

Clarity and colour of solution. A solution of the powder for injections equivalent to 0.5 g of Pentamidine isetionate in 10 ml of carbon-dioxide-free water R is clear and colourless. (Keep this solution for the "pH value".)

pH value. pH of the solution prepared above for the test of clarity and colour, 4.5–6.5.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6, activated at 105 °C for 1 hour, as the coating substance (a precoated plate from a commercial source is suitable), and as the mobile phase the upper layer obtained by shaking together 10 volumes of water, 8 volumes of 1-butanol R, and 2 volumes of glacial acetic acid R. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) dissolve a quantity of the powder for injections equivalent to 0.1 g of Pentamidine isetionate in 2 ml of methanol R. For solution (B) dilute 1 ml of solution A to 200 ml with methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay

Mix the contents of 10 containers and carry out the assay as described.

Use Method A as described under 2.10 Determination of nitrogen, with a quantity of the powder for injections equivalent to about 0.4 g of Pentamidine isetionate, accurately weighed, and 9 ml of nitrogen-free sulfuric acid (~1760 g/l) TS.

Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 14.82 mg of $C_{19}H_{24}N_4O_2,2C_2H_6O_4S$.

PETHIDINI HYDROCHLORIDI COMPRESSI
PETHIDINE HYDROCHLORIDE TABLETS

Category. Opioid analgesic.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg, 100 mg.

Requirements

Comply with the monograph for "Tablets".

Pethidine hydrochloride tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{21}NO_2$,HCl stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. To a quantity of the powdered tablets equivalent to about 0.05 g of Pethidine hydrochloride add 20 ml of chloroform R, shake, and filter. Evaporate the filtrate to dryness and dry the residue under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury). Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the *reference spectrum* of pethidine hydrochloride.

B. To a quantity of the powdered tablets equivalent to about 0.2 g of Pethidine hydrochloride add 20 ml of water, shake, and filter. To 5 ml of the filtrate (keep the remaining filtrate for tests C and D) add 5 ml of trinitrophenol/ethanol TS and shake; a yellow, crystalline precipitate is produced. Filter, wash with water, and dry the crystals at 105 °C for 2 hours; melting temperature, about 190 °C.

C. Evaporate 1 ml of the filtrate from test B to dryness on a water-bath, dissolve the residue in 1 ml of formaldehyde/sulfuric acid TS, and heat gently; the colour of the solution turns to pink, changing to violet-red and showing a red fluorescence when held in front of a strong light.

D. Dilute 5 ml of the filtrate from test B with 5 ml of water; it yields the reactions described under 2.1 General identification tests as characteristic of chlorides.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture

of 9 volumes of acetone R and 1 volume of 2-phenoxyethanol R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber, and dry it in a current of air. Use the impregnated plate immediately, carrying out the chromatography in the same direction as the impregnation.

Shake together 100 volumes of light petroleum R1, 8 volumes of 2-phenoxyethanol R, and 1 volume of diethylamine R, allow to settle, and use this solution as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.1 g of Pethidine hydrochloride with 5 ml of water, filter, shake the filtrate with 0.5 ml of sodium hydroxide (~200 g/l) TS and 2 ml of ether R, allow the layers to separate, and use the upper layer. For solution (B) dilute 0.5 ml of solution A to 50 ml with ether R. After removing the plate from the chromatographic chamber, allow it to dry in air for 10 minutes, return it to the chromatographic chamber, and repeat the development. Remove the plate, allow it to dry in air for 10 minutes, and spray with dichlorofluorescein TS. Allow to stand for 5 minutes and spray with water until the background is white to pale yellow.

Examine the chromatogram in daylight. The chromatogram shows red to orange spots.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Examine the chromatogram without delay in ultraviolet light (365 nm). The chromatogram shows spots with intense yellow fluorescence.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powdered tablets equivalent to about 0.5 g of Pethidine hydrochloride add 40 ml of water, 2.0 ml of sodium hydroxide (~200 g/l) TS, and extract immediately with quantities of 25 ml, 10 ml, and 10 ml of chloroform R. Wash each extract with the same 15 ml of water and filter into a dry flask. To the combined extracts, which should be clear and free from droplets of water, add 0.15 ml of 1-naphtholbenzein/ acetic acid TS and titrate with perchloric acid (0.05 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.05 mol/l) VS is equivalent to 14.19 mg of $C_{15}H_{21}NO_2,HCl$.

PHENOBARBITALI COMPRESSI
PHENOBARBITAL TABLETS

Category. Antiepileptic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 15–100 mg.

Requirements

Comply with the monograph for "Tablets".

Phenobarbital tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{12}H_{12}N_2O_3$ stated on the label.

Identity tests

- Either tests A, C, and D or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 0.4 g of Phenobarbital add 10 ml of dehydrated ethanol R, shake, and filter. Evaporate the filtrate to dryness and dry the residue at 105 °C for 1 hour. Use the residue for the following tests.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenobarbital RS or with the *reference spectrum* of phenobarbital. If the spectra obtained are not concordant, heat the residue in a sealed tube at 105 °C for 1 hour and prepare a new spectrum of the residue.

B. Melting temperature of the residue, about 174 °C.

C. Dissolve 20 mg of the residue in 5 ml of methanol R, add 1 drop of cobalt(II) chloride (30 g/l) TS and 3–4 drops of ammonia (~100 g/l) TS; a violet colour is produced.

D. To 0.20 g of the residue add about 2 ml of sulfuric acid (~1760 g/l) TS, 20 mg of sodium nitrate R, and allow to stand for 30 minutes; a yellow colour is produced.

Assay. Weigh and powder 20 tablets. To a quantity of the powdered tablets equivalent to about 0.2 g of Phenobarbital add 40 ml of methanol R and 15 ml of a freshly prepared solution of 30 mg of anhydrous sodium carbonate R per ml. Titrate with silver nitrate (0.1 mol/l) VS, determining the end-point potentiometrically.

Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

Disintegration test. Complies with the 5.3 Disintegration test for tablets and capsules. Time period: 30 minutes.

PHENOXYMETHYLPENICILLINI KALICI COMPRESSI

PHENOXYMETHYLPENICILLIN POTASSIUM TABLETS

Category. Antibacterial drug.

Storage. Phenoxymethylpenicillin potassium tablets should be kept in a tightly closed container.

Labelling. The designation on the container indicates the quantity of active ingredient in terms of the equivalent amount of phenoxymethylpenicillin. It also indicates whether the tablets are to be chewed before swallowing. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg.

Requirements

Comply with the monograph for "Tablets".

Phenoxymethylpenicillin potassium tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{16}H_{18}N_2O_5S$ stated on the label.

Identity tests

A. Shake a quantity of the powdered tablets equivalent to 80 mg of Phenoxymethylpenicillin potassium with water, dilute to 250 ml with water, and filter. The absorption spectrum of the filtrate, when observed between 230 nm and 350 nm, exhibits maxima at 268 nm and 274 nm and a minimum at 272 nm.

B. Shake a quantity of the powdered tablets equivalent to 1 g of Phenoxymethylpenicillin potassium with 5 ml of water, filter, and add 3 drops of hydrochloric acid (~70 g/l) TS. Mix, cool in ice, add 1 drop of sodium nitrite (100 g/l) TS and a solution composed of 50 mg of 2-naphthol R dis-

solved in a mixture of 2 ml of sodium hydroxide (~80 g/l) TS and 3 ml of water; no scarlet-red precipitate is formed (absence of procaine penicillin).

C. Ignite a quantity of the powdered tablets equivalent to 0.5 g of Phenoxymethylpenicillin potassium, add 5 ml of hydrochloric acid (~70 g/l) TS, boil, cool, and filter; the filtrate made alkaline yields the reaction described under 2.1 General identification tests as characteristic of potassium.

Loss on drying. Dry a quantity of the powdered tablets equivalent to 0.1 g of Phenoxymethylpenicillin potassium under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) at 60 °C for 3 hours; it loses not more than 15 mg/g.

Phenoxyacetic acid. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 90 volumes of diisopropyl ether R, 7 volumes of anhydrous formic acid R, and 1 volume of water as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.25 g of Phenoxymethylpenicillin potassium with 25 ml of a mixture of equal volumes of methanol R and water, filter, and use the clear filtrate. For solution (B) dissolve 10 mg of phenoxyacetic acid R in the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air and spray with a mixture of 0.15 g of potassium permanganate R dissolved in 100 ml of sulfuric acid (0.5 mol/l) VS. Examine the chromatogram in daylight.

Any spot corresponding to phenoxyacetic acid obtained with solution A is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powder equivalent to about 125 mg of Phenoxymethylpenicillin potassium, accurately weighed, with 300 ml of water for 30 minutes, add sufficient water to produce 500 ml, dilute 25 ml to 100 ml with water, and filter. Transfer two 2-ml aliquots of the filtrate into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place it in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*. From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{16}H_{18}N_2O_5S$ in the sample being examined by comparison with phenoxymethylpenicillin potassium RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.63 ± 0.03.

Dissolution. For tablets other than those that are intended to be chewed before swallowing, carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 500 ml of dissolution buffer, pH 6.8, TS and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of about 5 ml of the medium directly through an in-line filter. Measure the absorbance of the filtered sample, suitably diluted if necessary, at the maximum at 268 nm. At the same time measure the absorbance at the maximum at 278 nm of a suitable solution of phenoxymethylpenicillin potassium RS in dissolution buffer, pH 6.8, TS.

For each of the six tablets tested, calculate the total amount of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, in the medium from the absorbances obtained and from the declared content of $C_{16}H_{18}N_2O_5S$ in phenoxymethylpenicillin potassium RS. The average amount of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$, in solution is not less than 85% of the amount declared on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 85%.

PHENYTOINI NATRICI COMPRESSI
PHENYTOIN SODIUM TABLETS

Category. Antiepileptic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 25 mg, 50 mg, 100 mg.

Requirements

Comply with the monograph for "Tablets".

Phenytoin sodium tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{11}N_2NaO_2$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 0.1 g of Phenytoin sodium add 20 ml of water, shake, and filter. Acidify the filtrate with hydrochloric acid (~70 g/l) TS and extract with chloroform R. Wash the chloroform extract with water, dry with anhydrous sodium sulfate R, and evaporate to dryness. Use the residue for tests A and B.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from phenytoin RS or with the *reference spectrum* of phenytoin.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 9 volumes of chloroform R and 1 volume of acetone R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions in chloroform R. For solution (A) use 1 mg of the residue per ml. For solution (B) use 1 mg of phenytoin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powdered tablets equivalent to about 40 mg of Phenytoin sodium add 2.0 ml of ammonia (~100 g/l) TS and heat until boiling begins. Add 1 drop of copper(II) sulfate (160 g/l) TS and shake; a blue-violet solution with a blue-green precipitate is produced. Allow to stand for 3 minutes, filter, and wash with water; pink needles remain on the filter.

D. To a quantity of the powdered tablets equivalent to about 40 mg of Phenytoin sodium add 5 ml of water, shake, and filter. The filtrate yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Benzophenone. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 75 volumes of hexane R and 30 volumes of dioxan R as the mobile phase. Apply separately to the plate 5 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.1 g of Phenytoin sodium with 5 ml of methanol R, warm on a water-bath while shaking, filter, and use the filtrate. For solution (B) use 0.10 mg of benzophenone R per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot corresponding to benzophenone obtained with solution A is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. Transfer to a separatory funnel a quantity of the powdered tablets equivalent to about 0.3 g of Phenytoin sodium, add 25 ml of water, and shake. Add 50 ml of ether R, shake, and add 10 drops of bromophenol blue/ethanol TS. Titrate with hydrochloric acid (0.1 mol/l) VS, shaking vigorously, until the colour of the aqueous layer turns to bluish-grey. Transfer the aqueous layer to a stoppered conical flask. Wash the ether layer

with 5 ml of water and combine the washing with the aqueous layer in the conical flask. Add 20 ml of ether R and continue the titration with hydrochloric acid (0.1 mol/l) VS, shaking vigorously, until the colour of the aqueous layer turns to pale green.

Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 27.43 mg of $C_{15}H_{11}N_2NaO_2$.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms.

PIPERAZINI ADIPATIS COMPRESSI
PIPERAZINE ADIPATE TABLETS

Category. Intestinal anthelminthic drug.

Additional information. Strength in the current WHO Model list of essential medicines: the equivalent of 500 mg of piperazine hydrate.

600 mg of piperazine adipate is approximately equivalent to 500 mg of piperazine hexahydrate.

Requirements

Comply with the monograph for "Tablets".

Piperazine adipate tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_4H_{10}N_2,C_6H_{10}O_4$ stated on the label.

Identity tests

A. See the test described below under "Related substances". Examine the chromatograms after spraying with the two triketohydrindene hydrate reagent solutions. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

B. Shake a quantity of the powdered tablets equivalent to 1 g of Piperazine adipate with 20 ml of water for 5 minutes and filter. To 10 ml of the filtrate (keep the remaining filtrate for test C) add 5 ml of hydrochloric acid (~250 g/l) TS and allow to stand for 10 minutes; a crystalline precipitate is formed. Filter, wash the precipitate 2–3 times with 3-ml portions of cold water, and dry at 105 °C; melting temperature, about 152 °C (adipic acid).

C. To the remaining filtrate obtained in test B add 0.5 g of sodium nitrite R. Heat to boiling and cool in ice for 15 minutes, stirring if necessary to induce crystallization. Filter, wash with 10 ml of ice-water, and dry the precipitate at 105 °C; melting temperature, about 158 °C (N,N'-dinitrosopiperazine).

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a freshly prepared mixture of 2 volumes of ammonia (~260 g/l) TS and 8 volumes of acetone R as the mobile phase. Prepare 500 ml of the following mixture of solvents for solutions B, C, D, E, and F: mix 2 volumes of ethanol (~750 g/l) TS with 3 volumes of ammonia (~260 g/l) TS. Apply separately to the plate 5 µl of each of the following six solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 1 g of Piperazine adipate with a mixture of 6 ml of ammonia (~260 g/l) TS diluted to 10 ml with water, filter, and use the clear filtrate. For solution (B) dilute 1 ml of solution A to 10 ml with the mixture of solvents. For solution (C) dissolve 0.1 g of piperazine adipate RS in sufficient mixture of solvents to produce 10 ml. For solution (D) dissolve 25 mg of ethylenediamine R in sufficient mixture of solvents to produce 100 ml. For solution (E) dissolve 25 mg of triethylenediamine R in sufficient mixture of solvents to produce 100 ml. For solution (F) dissolve 12.5 mg of triethylenediamine R in 5 ml of test solution A and dilute to 50 ml with the mixture of solvents. After removing the plate from the chromatographic chamber, allow to dry at 105 °C and spray successively with a 3 mg/ml solution of triketohydrindene hydrate R dissolved in a mixture of 3 volumes of glacial acetic acid R and 100 volumes of 1-butanol R and then with a 1.5 mg/ml solution of triketohydrindene hydrate R in ethanol (~750 g/l) TS. Dry the plate at 105 °C for 10 minutes and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D. Spray the plate with iodine (0.05 mol/l) VS, allow to stand for 10 minutes, and examine the chromatogram in daylight.

Any spot corresponding to triethylenediamine obtained with solution A is not more intense than that obtained with solution E. The test is valid only if the chromatogram obtained with solution F shows two clearly separated spots. Disregard any spot remaining on the line of application.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.2 g of Piperazine adipate, accurately weighed, add 10 ml of water and shake for 1 hour. Filter and wash the residue with two portions, each of 10 ml, of water. To the combined extract and washings add 5 ml of sulfuric acid (~1760 g/l) TS and 100 ml of trinitrophenol (7 g/l) TS, heat on a water-bath for 15 minutes, and allow to stand for 1 hour. Filter, wash the residue with successive quantities of trinitrophenol (7 g/l) TS, using 10 ml each time, and

finally wash with dehydrated ethanol R. Dry the residue to constant mass at 105 °C.

Each g of residue is equivalent to 426.8 mg of $C_4H_{10}N_2,C_6H_{10}O_4$.

PIPERAZINI CITRATIS COMPRESSI
PIPERAZINE CITRATE TABLETS

Category. Intestinal anthelminthic drug.

Storage. Piperazine citrate tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Requirements

Comply with the monograph for "Tablets".

Piperazine citrate tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $(C_4H_{10}N_2)_3,2C_6H_8O_7$ stated on the label.

Identity tests

A. See the test described below under "Related substances". Examine the chromatograms after spraying with the two triketohydrindene hydrate reagent solutions. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

B. Mix a quantity of the powdered tablets equivalent to 0.2 g of Piperazine citrate with 5 ml of hydrochloric acid (~70 g/l) TS and filter. To the filtrate add 0.5 g of sodium nitrite R and shake to dissolve. Cool in ice for 15 minutes, stir if necessary to induce crystallization, filter, wash with 10 ml of ice-water, and dry the precipitate at 105 °C; melting temperature, about 158 °C (N,N'-dinitrosopiperazine).

C. To a quantity of the powdered tablets equivalent to 0.5 g of Piperazine citrate add 10 ml of water, shake, and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of citrates.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a freshly prepared mixture of 2 volumes of ammonia (~260 g/l) TS and 8 volumes of acetone

R as the mobile phase. Prepare 500 ml of the following mixture of solvents for solutions B, C, D, E, and F: mix 2 volumes of ethanol (~750 g/l) TS with 3 volumes of ammonia (~260 g/l) TS. Apply separately to the plate 5 µl of each of the following six solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 1 g of Piperazine citrate with a mixture of 6 ml of ammonia (~260 g/l) TS diluted to 10 ml with water, filter, and use the clear filtrate. For solution (B) dilute 1 ml of solution A to 10 ml with the mixture of solvents. For solution (C) dissolve 0.1 g of piperazine citrate RS in sufficient mixture of solvents to produce 10 ml. For solution (D) dissolve 25 mg of ethylenediamine R in sufficient mixture of solvents to produce 100 ml. For solution (E) dissolve 25 mg of triethylenediamine R in sufficient mixture of solvents to produce 100 ml. For solution (F) dissolve 12.5 mg of triethylenediamine R in 5 ml of test solution A and dilute to 50 ml with the mixture of solvents. After removing the plate from the chromatographic chamber, allow to dry at 105 °C and spray successively with a 3 mg/ml solution of triketohydrindene hydrate R dissolved in a mixture of 3 volumes of glacial acetic acid R and 100 volumes of 1-butanol R and then with a 1.5 mg/ml solution of triketohydrindene hydrate R in ethanol (~750 g/l) TS. Dry the plate at 105 °C for 10 minutes, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D. Spray the plate with iodine (0.05 mol/l) VS, allow to stand for 10 minutes, and examine the chromatogram in daylight.

Any spot corresponding to triethylenediamine obtained with solution A is not more intense than that obtained with solution E. The test is valid only if the chromatogram obtained with solution F shows two clearly separated spots. Disregard any spot remaining on the line of application.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.2 g of Piperazine citrate, accurately weighed, add 10 ml of a mixture of 1 part of hydrochloric acid (~70 g/l) TS and 3 parts of water, and shake for 1 hour. Filter and wash the residue with two portions, each of 10 ml, of water. To the combined extract and washings add 100 ml of trinitrophenol (7 g/l) TS, heat on a water-bath for 15 minutes, and allow to stand for 1 hour. Filter, wash the residue with successive quantities of trinitrophenol (7 g/l) TS, using 10 ml each time, and finally wash with dehydrated ethanol R. Dry the residue to constant mass at 105 °C.

Each g of residue is equivalent to 393.5 mg of $(C_4H_{10}N_2)_3,2C_6H_8O_7$.

PRAZIQUANTELI COMPRESSI
PRAZIQUANTEL TABLETS

Category. Anthelminthic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 150 mg, 600 mg.

Requirements

Comply with the monograph for "Tablets".

Praziquantel tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{19}H_{24}N_2O_2$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 0.1 g of Praziquantel add 10 ml of chloroform R, shake, and filter. Evaporate the filtrate to dryness and dry the residue at 50 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury). Use the residue for tests A and D.

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from praziquantel RS or with the *reference spectrum* of praziquantel.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. The absorption spectrum of the solution obtained in the "Assay", when observed between 230 nm and 350 nm, exhibits two maxima at about 264 nm and 272 nm.

D. Melting temperature of the residue, about 138 °C.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 85 volumes of toluene R and 15 volumes of methanol R as the mobile phase. Apply separately to the plate, in a current of nitrogen R, 10 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.25 g of Praziquantel with 5 ml of chloroform R, filter, and

use the filtrate. For solution (B) use 0.05 g of praziquantel RS per ml of chloroform R. Then apply 2 µl of each of the following 2 solutions in chloroform R containing (C) 0.5 mg of praziquantel RS per ml, and (D) 1.0 mg of praziquantel RS per ml. Allow the mobile phase to ascend 7 cm. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, place in a chamber with iodine vapours, and allow to stand for 20 minutes. Examine the chromatogram immediately in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C, except one spot above the main spot which is not more intense than that obtained with solution D.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 25 mg of Praziquantel add 50 ml of ethanol (~750 g/l) TS, shake, and dilute to volume with the same solvent. Filter and discard the first 5 ml of the filtrate. Measure the absorbance of a 1-cm layer at the maximum at about 264 nm against a solvent cell containing ethanol (~750 g/l) TS. Calculate the percentage content of $C_{19}H_{24}N_2O_2$ by comparison with a solution containing 0.50 mg of praziquantel RS per ml of ethanol (~750 g/l) TS.

PREDNISOLONI COMPRESSI
PREDNISOLONE TABLETS

Category. Adrenal hormone.

Additional information. Strength in the current WHO Model list of essential medicines: 1 mg, 5 mg.

Requirements

Comply with the monograph for "Tablets".

Prednisolone tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{21}H_{28}O_5$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

To a quantity of the powdered tablets equivalent to about 0.05 g of Prednisolone add 10 ml of acetone R, shake, and filter. Evaporate the filtrate to dryness and use the residue for the "Identity tests" and "Related substances".

A. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone RS or with the *reference spectrum* of prednisolone.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using kieselguhr R1 as the coating substance and a mixture of 9 volumes of acetone R and 1 volume of formamide R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use chloroform R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the residue per ml, and (B) 2.5 mg of prednisolone RS per ml. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes, and spray the hot plate with sulfuric acid/ethanol TS. Heat at 120 °C for a further 10 minutes, allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To 5 mg of the residue add 1.0 ml of ethanol (~750 g/l) TS and shake. Then add 1.0 ml of potassio-cupric tartrate TS and heat to boiling; an orange precipitate is produced slowly.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 77 volumes of dichloromethane R, 15 volumes of ether R, 8 volumes of methanol R, and 1.2 volumes of water as the mobile phase. Apply separately to the plate 1 µl of each of 2 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 15 mg of the residue per ml, and (B) 0.30 mg of the residue per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and heat at 105 °C for 10 minutes. Cool, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powdered tablets equivalent to about 20 mg of Prednisolone, add 15 ml of water, shake with four

quantities, each of 25 ml, of chloroform R, and filter the chloroform layer through cotton wool previously washed with chloroform R. Add sufficient chloroform R to the filtrate to produce 250 ml and dilute 25 ml to 100 ml with the same solvent. Transfer 10 ml of the resulting solution to a glass-stoppered, 50-ml conical flask, carefully evaporate to dryness, and dissolve the residue in 20 ml of dehydrated ethanol R. Transfer 20 ml of dehydrated ethanol R to a similar flask to serve as the blank. To each of the flasks add 2.0 ml of blue tetrazolium/ethanol TS and mix. Then add to each flask 2.0 ml of tetramethylammonium hydroxide/ethanol TS, mix, and allow to stand in the dark for 90 minutes. Measure the absorbance of a 1-cm layer at the maximum at about 525 nm against a solvent cell containing the blank.

Calculate the percentage content of $C_{21}H_{28}O_5$ by comparison with prednisolone RS, similarly and concurrently examined.

Uniformity of content

For tablets containing 1 mg of Prednisolone. Individually transfer 10 powdered tablets to 10 separate 100-ml volumetric flasks, add 50 ml of ethanol (~750 g/l) TS, shake, and dilute to volume with the same solvent.

For tablets containing 5 mg of Prednisolone. Individually transfer 10 powdered tablets to 10 separate 100-ml volumetric flasks, add 50 ml of ethanol (~750 g/l) TS, shake, and dilute to volume with the same solvent. Dilute 2.0 ml to 10 ml with ethanol (~750 g/l) TS. Measure the absorbance of a 1-cm layer of the solutions at the maximum at about 242 nm.

Calculate the tablet content of $C_{21}H_{28}O_5$ in mg by comparison with prednisolone RS. The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations.

PREDNISOLONI ET NATRII PHOSPHATIS INJECTIO

PREDNISOLONE SODIUM PHOSPHATE INJECTION

Description. A colourless solution.

Category. Adrenal hormone.

Storage. Prednisolone sodium phosphate injection should be protected from light and stored at a temperature not exceeding 15 °C.

Labelling. The designation on the container should state the amount of active ingredient as the equivalent quantity of prednisolone in a suitable dose volume. It should also state whether any buffering agent is added.

Additional information. Strength in the current WHO Model list of essential medicines: 20 mg, 25 mg in vials.

Prednisolone sodium phosphate injection contains a suitable stabilizing agent, and may contain a suitable buffering agent. The solution is sterilized by "Filtration" or by another suitable method (see 5.8 Methods of sterilization).

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Prednisolone sodium phosphate injection is a sterile solution of prednisolone sodium phosphate in water for injections.

Prednisolone sodium phosphate injection contains not less than **90.0%** and not more than **110.0%** of the equivalent amount of prednisolone $C_{21}H_{28}O_5$ stated on the label.

Identity tests

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and 6 volumes of 1-butanol R, 2 volumes of acetic anhydride R, and 2 volumes of water as the mobile phase, prepared immediately before use. Apply separately to the plate 5 µl of each of the following four solutions. For solution (A) dilute a volume of the injection to obtain a concentration of the equivalent of 2 mg of prednisolone per ml. For solution (B) dissolve 27 mg of prednisolone sodium phosphate RS in 10 ml of water. For solution (C) prepare a mixture of equal volumes of solutions A and B. For solution (D) mix 2 ml of solution B with 2 ml of a solution containing 29 mg of betamethasone sodium phosphate RS in 10 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air until the odour of solvent is no longer perceptible, heat to 110 °C for 10 minutes, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The chromatogram obtained with solution D shows two principal spots with almost the same R_f values. Additional spots due to pharmaceutical aids may be observed in the chromatograms obtained with solutions A and C.

B. Evaporate a volume of the injection equivalent to 2 mg of prednisolone to dryness on a water-bath. Dissolve the residue in 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 2 minutes; a red colour is produced.

pH value. pH of the injection, 7.0–9.0.

Assay. Dilute an accurately measured volume of the injection equivalent to about 20 mg of prednisolone with sufficient water to produce 200 ml. To 25 ml of this solution add 2.5 g of sodium chloride R and 1 ml of hydrochloric acid (0.1 mol/l) VS, mix, and shake with three quantities, each of 25 ml, of chloroform R. Wash each chloroform extract with 1 ml of hydrochloric acid (0.1 mol/l) VS, add the washings to the aqueous solution, and discard the chloroform layers. Extract the aqueous solution with two quantities, each of 10 ml, of tributyl phosphate R and dilute the combined extracts to 25 ml with methanol R. Transfer 2 ml to a stoppered tube and add 10 ml of isoniazid TS, heat to 50 °C for 3 hours, protecting the solution from light, and cool.

Measure the absorbance in a 1-cm layer at the maximum at about 405 nm. Similarly prepare a blank solution, omitting the injection to be examined. Repeat the procedure using 25 ml of a solution of prednisolone sodium phosphate RS containing the equivalent of 0.10 mg/ml of prednisolone. Determine the latter by diluting an aliquot with water, measuring the absorbance of the dilution at the maximum at about 247 nm. Calculate the content of $C_{21}H_{28}O_5$ in the injection being examined by comparison with the absorbances obtained and the exact strength of the solution of prednisolone sodium phosphate RS, using 419 as the $A^{1\%}_{1\,cm}$ at the maximum at 247 nm.

PREDNISOLONI ET NATRII SUCCINATIS PULVIS AD INJECTIONEM

PREDNISOLONE SODIUM SUCCINATE POWDER FOR INJECTIONS

Description. A white powder or friable lumps; odourless.

Category. Adrenal hormone.

Labelling. The designation on the container should state the dose as the equivalent amount of prednisolone. It should also state the nature of the buffering agent. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 20 mg, 25 mg in vials.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Prednisolone sodium succinate powder for injections is a sterile powder of prednisolone sodium succinate prepared from prednisolone succinate with the aid of sodium hydroxide or sodium carbonate.

Prednisolone sodium succinate powder for injections usually contains a suitable buffering agent. The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

The container of Prednisolone sodium succinate powder for injections contains not less than **90.0%** and not more than **110.0%** of the equivalent amount of prednisolone $C_{21}H_{28}O_5$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Dissolve a quantity of the powder for injections equivalent to 40 mg of prednisolone in 20 ml of water, add 3 ml of hydrochloric acid (~70 g/l) TS, and extract with 25 ml of chloroform R. Filter the extract into a beaker, evaporate to dryness on a water-bath, and dry the residue at 60 °C for 1 hour. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone succinate RS or with the *reference spectrum* of prednisolone succinate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and 6 volumes of 1-butanol R, 2 volumes of acetic anhydride R, and 2 volumes of water as the mobile phase, prepared immediately before use. Apply separately to the plate 5 µl of each of the following two solutions. For solution (A) dissolve a quantity of the powder for injections to obtain a concentration equivalent to 2 mg of prednisolone per ml. For solution (B) dissolve 28 mg of prednisolone succinate RS in 10 ml of water. After removing the plate from the chromatographic chamber, allow it to dry in air until the odour of solvent is no longer perceptible, heat to 110 °C for 10 minutes, and examine the chromatogram in ultraviolet light (254 nm).

   The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. To a quantity of the powder for injections equivalent to 2 mg of prednisolone add 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 2 minutes; a red colour is produced.

D. Ignite a quantity of the powder for injections equivalent to 20 mg of prednisolone and dissolve the residue in acetic acid (~60 g/l) TS. The solution yields reaction B described under 2.1 General identification tests as characteristic of sodium.

Clarity of solution. A solution containing a quantity of the powder for injections equivalent to 0.35 g of prednisolone in 10 ml of carbon-dioxide-free water R is clear. (Keep this solution for the "pH value".)

Loss on drying. Dry the powder for injections at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 20 mg/g.

pH value. pH of the solution prepared above for the test of clarity, 6.5–8.0.

Assay

Mix the contents of 10 containers and carry out the assay as described.

Dissolve a portion of the powder for injections equivalent to about 0.05 g of prednisolone, accurately weighed, in 5 ml of water and dilute with sufficient ethanol (~750 g/l) TS to produce 200 ml. Dilute 4 ml to 100 ml with ethanol (~750 g/l) TS. Transfer 20 ml of the resulting solution to a glass-stoppered 50-ml conical flask (*solution A*). Separately, dissolve 64 mg of prednisolone succinate RS, accurately weighed, in 100 ml of ethanol (~750 g/l) TS, add 5 ml of water, and dilute with sufficient ethanol (~750 g/l) TS to produce 200 ml. Dilute 4 ml to 100 ml with ethanol (~750 g/l) TS. Transfer 20 ml of the resulting solution to a glass-stoppered 50-ml conical flask (*solution B*). To each flask containing *solutions A* and *B* and a third one containing 20 ml of ethanol (~750 g/l) TS to serve as a blank, add 2 ml of blue tetrazolium/ethanol TS, mix, then add 2 ml of tetramethylammonium hydroxide/ethanol TS, mix, and allow to stand in the dark for 90 minutes.

Without delay measure the absorbance of *solutions A* and *B* against the blank, using a suitable spectrophotometer at a maximum wavelength of about 525 nm.

Calculate the amount of $C_{21}H_{28}O_5$ in the substance being examined using the formula $5C(0.7827)(A_u/A_s)$ where C is the concentration in mg per ml of prednisolone succinate RS, 0.7827 is the ratio of the relative molecular mass of prednisolone to that of prednisolone succinate, and A_u and A_s are the absorbances of *solutions A* and *B*, respectively.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 5.8 IU of endotoxin RS per mg of prednisolone.

PRIMAQUINI DIPHOSPHATIS COMPRESSI
PRIMAQUINE DIPHOSPHATE TABLETS

Category. Antimalarial drug.

Labelling. The designation on the container indicates the quantity of active ingredient in terms of the equivalent amount of primaquine.

Additional information. Strength in the current WHO Model list of essential medicines: 7.5 mg and 15 mg of primaquine base.

The tablets may be coated.

Requirements

Comply with the monograph for "Tablets".

Primaquine diphosphate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{21}N_3O$ stated on the label.

Identity tests

- Either tests A and D or tests B, C, and D may he applied.

A. To a quantity of the powdered tablets equivalent to 60 mg of Primaquine add a mixture of 10 ml of water and 2 ml of sodium hydroxide (~80 g/l) TS, and shake with two 20-ml quantities of chloroform R. Wash the chloroform extracts with water, dry with anhydrous sodium sulfate R, and evaporate to dryness. Dissolve the residue in 2 ml of chloroform R and carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from primaquine diphosphate RS similarly treated or with the *reference spectrum* of primaquine.

B. Shake a quantity of the powdered tablets equivalent to 15 mg of Primaquine with 100 ml of hydrochloric acid (0.01 mol/l) VS and filter. The absorption spectrum of this solution, when observed between 310 nm and 450 nm, exhibits two maxima at about 332 nm and 415 nm. The absorbances of a 1-cm layer at these wavelengths are between 45 and 52, and 27 and 35,

respectively. Dilute 5 ml of the solution to 50 ml with hydrochloric acid (0.01 mol/l) VS. The absorption spectrum of this solution, when observed between 215 nm and 310 nm, exhibits three maxima at about 225 nm, 265 nm, and 282 nm. The absorbances of a 1-cm layer at these wavelengths are between 495 and 515, 335 and 350, and 330 and 345, respectively.

C. Shake a quantity of the powdered tablets equivalent to 25 mg of Primaquine with 10 ml of water and filter. To 2 ml of the filtrate add 3 ml of water and 1 ml of ceric ammonium sulfate/nitric acid TS; a deep violet colour is immediately produced. (Keep the remainder of the filtrate for test D.)

D. To the filtrate remaining from test C add 3 ml of nitric acid (~130 g/l) TS; it yields reaction A described under 2.1 General identification tests as characteristic of orthophosphates.

Assay. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.15 g of Primaquine, accurately weighed, to a separatory funnel, add 20 ml of water and 5 ml of sodium hydroxide (~80 g/l) TS, and extract with four 25-ml quantities of chloroform R. Evaporate the combined chloroform extracts to a volume of about 10 ml, add 40 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 12.97 mg of $C_{15}H_{21}N_3O$.

PROBENECIDI COMPRESSI
PROBENECID TABLETS

Category. Antigout drug.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Requirements

Comply with the monograph for "Tablets".

Probenecid tablets contain not less than **95.0%** and not more than **105.0%** of the amount of $C_{13}H_{19}NO_4S$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

Triturate a quantity of the powdered tablets equivalent to 0.5 g of Probenecid with ethanol (~750 g/l) TS, filter, concentrate the filtrate to a small volume by evaporation, cool, and filter. Recrystallize the residue from ethanol (~457 g/l) TS and use it for the following two tests:

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from probenecid RS or with the *reference spectrum* of probenecid.

B. Melting temperature of the residue, about 199 °C.

C. The absorption spectrum of the solution prepared for the assay, when observed between 220 nm and 300 nm, exhibits two maxima at about 225 nm and 248 nm; the absorbance of a 1-cm layer at the maximum of 248 nm is between 310 and 350.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 15 volumes of 1-propanol R and 3 volumes of ammonia (~17 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.2 g of Probenecid with 10 ml of a mixture of 1 volume of ammonia (~17 g/l) TS and 9 volumes of ethanol (~750 g/l) TS, centrifuge, and use the supernatant liquid. For solution (B) dilute 1 ml of solution A to 100 ml with the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.2 g of Probenecid, accurately weighed, add 200 ml of ethanol (~750 g/l) TS and 5 ml of hydrochloric acid (1 mol/l) VS. Heat on a water-bath at 70 °C for 30 minutes, shaking the flask occasionally, cool, add sufficient ethanol (~750 g/l) TS to produce 250 ml, and filter. To 5 ml of the filtrate, add 5 ml of hydrochloric acid (0.1 mol/l) VS, and dilute to 250 ml with ethanol (~750 g/l) TS.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 248 nm and calculate the content of $C_{13}H_{19}NO_4S$, using the absorptivity value of 33.2 ($A^{1\%}_{1\,cm}$ = 332).

Disintegration test. Complies with the test for 5.3 Disintegration test for tablets and capsules. Time period: 30 minutes.

PROCAINI BENZYLPENICILLINI PULVIS AD INJECTIONEM

PROCAINE BENZYLPENICILLIN POWDER FOR INJECTIONS

Description. A white or almost white, crystalline powder.

Category. Antibacterial drug.

Storage. Procaine benzylpenicillin powder for injections should be protected from light and stored at a temperature not exceeding 25 °C.

Labelling. The designation on the container should state the nature of the buffering agent. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 1 g and 3 g.

Requirements

The powder for injections and the reconstituted preparation for injections comply with the monograph for "Parenteral preparations".

Definition. Procaine benzylpenicillin powder for injections is a sterile powder of procaine benzylpenicillin.

Procaine benzylpenicillin powder for injections usually contains a suitable buffering agent. The powder is sterilized by a suitable method (see 5.8 Methods of sterilization). The reconstituted preparation is a suspension.

The container of Procaine benzylpenicillin powder for injections contains not less than **90.0%** and not more than **110.0%** of the amount of total penicillins calculated as $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$ and not less than **36.0%** and not more than **44.0%** of $C_{13}H_{20}N_2O_2$ both stated on the label.

Identity tests

A. To a quantity of the powder for injections equivalent to 2 mg of Procaine benzylpenicillin in a test-tube add 0.05 ml of water followed by 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is almost colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains almost colourless. Place the same quantity of the powder for injections in a second test-tube, add 1 drop of water and 2 ml of formaldehyde/sulfuric acid TS, and mix; the solution is almost colourless and after a few minutes the

colour changes to yellow-brown. Immerse the test-tube for 1 minute in a water-bath; a reddish brown colour is produced.

B. Dissolve a quantity of the powder for injections equivalent to 10 mg of Procaine benzylpenicillin in 10 ml of water and add 0.5 ml of neutral red/ethanol TS. Add sufficient sodium hydroxide (0.01 mol/l) VS to give a permanent orange colour and then add 1 ml of penicillinase TS; the colour changes rapidly to red.

C. A quantity of the powder for injections equivalent to 50 mg of Procaine benzylpenicillin yields the reaction described for the identification of primary aromatic amines under 2.1 General identification tests, producing a bright, orange-red precipitate.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using a quantity of the powder for injections equivalent to 0.5 g of Procaine benzylpenicillin; the water content is not less than 28 mg/g and not more than 42 mg/g.

Assays

Mix the contents of 10 containers and carry out the assays as described.

Total penicillins. Suspend a quantity of the powder for injections equivalent to about 0.045 g of Procaine benzylpenicillin, accurately weighed, in sufficient water to produce 1000 ml. Transfer two 2-ml aliquots of this suspension into separate stoppered tubes. To one tube add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tube, and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 ml of water and mix (*solution B*).

Without delay measure the absorbance of a 1-cm layer at the maximum at about 314 nm against a solvent cell containing a mixture of 2 ml of water and 10 ml of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B*, calculate the amount of $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$ in the substance being examined by comparison with 0.050 g of benzylpenicillin sodium RS, taking into account that each mg of benzylpenicillin sodium RS ($C_{16}H_{17}N_2NaO_4S$) is equivalent to 1.601 mg of $C_{16}H_{18}N_2O_4S$,$C_{13}H_{20}N_2O_2$. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be 0.62 ± 0.03.

Procaine. Suspend a quantity of the powder for injections equivalent to about 0.5 g of Procaine benzylpenicillin, accurately weighed, in 10 ml of water, add 5 ml of sodium carbonate (75 g/l) TS, and extract with four successive quantities, each of 25 ml, of chloroform R, filter the chloroform extracts, and evap-

orate to a small volume on a water-bath. Add 20 ml of hydrochloric acid (0.1 mol/l) VS and distil off the remaining chloroform. Cool, add 0.25 ml of methyl red/ethanol TS, and back-titrate with sodium hydroxide (0.1 mol/l) VS.

Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 23.63 mg of $C_{13}H_{20}N_2O_2$.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzylpenicillin.

PYRANTELI EMBONATIS COMPRESSI
PYRANTEL EMBONATE TABLETS

Category. Anthelminthic drug.

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg of pyrantel.

Requirements

Comply with the monograph for "Tablets".

Pyrantel embonate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{11}H_{14}N_2S,C_{23}H_{16}O_6$ stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.

To a quantity of the powdered tablets equivalent to about 0.05 g of Pyrantel embonate add a mixture of 10 ml of chloroform R, 10 ml of methanol R, and about 1 ml of ammonia (~260 g/l) TS, shake, and filter. Evaporate the filtrate to dryness on a water-bath, dissolve in a small volume of methanol R, and allow to recrystallize. Separate the crystals, dry at 80 °C for 2 hours, and use the dried crystals for the "Identity tests" and "Related substances".

A. Carry out the examination with the dried crystals as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from pyrantel embonate RS or with the *reference spectrum* of pyrantel embonate.

B. See the test described below under "Related substances". The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. Dissolve 5 mg of the dried crystals in 1.0 ml of hydrochloric acid (~70 g/l) TS and add 1.0 ml of formaldehyde/sulfuric acid TS; a violet-red colour is produced.

D. Dissolve about 2 mg of the dried crystals in 2 ml of sulfuric acid (~1760 g/l) TS; a yellow colour is produced which changes to orange and finally to red.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2 as the coating substance and a mixture of 20 volumes of ethyl acetate R, 5 volumes of methanol R, and 1.5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 100 ml of each of 3 solutions in a mixture of 5 volumes of chloroform R, 5 volumes of methanol R, and 0.5 volume of ammonia (~260 g/l) TS containing (A) 20 mg of the dried crystals per ml, (B) 20 mg pyrantel embonate RS per ml, and (C) 0.20 mg of the dried crystals per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 10 minutes, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C.

Assay

The operations described below must be carried out in subdued light and without any prolonged interruptions, preferably using low-actinic glassware.

Weigh and powder 20 tablets. To a quantity of the powdered tablets equivalent to about 0.1 g add 10 ml of dioxan R and 10 ml of ammonia (100 g/l) TS, and shake for 10 minutes. Dilute to 100 ml with perchloric acid (~140 g/l) TS, filter, discard the first 10 ml of the filtrate, and transfer 5 ml of the subsequent filtrate to a 50-ml volumetric flask. Dilute to volume with perchloric acid (~140 g/l) TS and mix. Transfer 25 ml to a 250-ml separatory funnel, add 100 ml of chloroform R, and shake well. Drain off the chloroform layer into a second separatory funnel. Repeat the extraction of the aqueous phase with a second 100-ml portion of chloroform R, and combine the chloroform extracts into the same separatory funnel. Add 40 ml of hydrochloric acid (0.05 mol/l) VS to the combined chloroform extracts and shake well. Drain off the chloroform phase into a third separatory funnel and extract with a further 40-ml portion of hydrochloric acid (0.05 mol/l) VS, discarding the chloroform phase. Combine the aqueous phases in a 100-ml volumetric flask, rinse the separatory funnel draining into the volumetric flask, and dilute to volume with hydrochloric acid (0.05 mol/l) VS. Measure the absorbance of a 1-cm layer at the maximum at about 311 nm against a solvent cell containing hydrochloric acid (0.05 mol/l) VS.

Calculate the percentage content of $C_{11}H_{14}N_2S,C_{23}H_{16}O_6$ by comparison with pyrantel embonate RS, similarly and concurrently examined.

PYRAZINAMIDI COMPRESSI
PYRAZINAMIDE TABLETS

Category. Antituberculosis drug.

Additional information. Strength in the current WHO Model list of essential medicines: 500 mg.

Requirements

Comply with the monograph for "Tablets".

Pyrazinamide tablets contain not less than **93.0%** and not more than **107.0%** of the amount of $C_5H_5N_3O$ stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Shake a quantity of the powdered tablets equivalent to 0.25 g of Pyrazinamide with 20 ml of dehydrated ethanol R, filter, and evaporate the filtrate to dryness. Dry the residue at 105 °C for 30 minutes and carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum of the residue is concordant with the spectrum obtained from pyrazinamide RS or with the *reference spectrum* of pyrazinamide.

B. Shake a quantity of the powdered tablets equivalent to 0.050 g of Pyrazinamide with 50 ml of water and filter. Dilute 1 ml of the filtrate to 100 ml with water. The absorption spectrum of this solution, when observed between 230 nm and 350 nm, exhibits two maxima at about 268 nm and 310 nm. The ratio of the absorbance of a 1-cm layer at 268 nm to that at 310 nm is between 11.6 and 12.0.

C. To a quantity of the powdered tablets equivalent to 0.06 g of Pyrazinamide add 5 ml of sodium hydroxide (~80 g/l) TS and heat on a water-bath; vapours are evolved. Insert moistened pH-indicator paper R into the vapours; the colour of the paper changes to an alkaline range, and an odour of ammonia is perceptible.

Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 6 volumes of 1-butanol R, 2 volumes of glacial acetic acid R, and 2 volumes of water as the mobile phase, but allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 20 µl of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets equivalent to 0.1 g of Pyrazinamide with 50 ml of a mixture of 9 volumes of chloroform R and 1 volume of methanol R, and filter. Evaporate the filtrate to dryness and dissolve the residue in sufficient mixture of solvents to produce 10 ml. For solution (B) dilute 1 volume of solution A to 500 volumes with the same mixture of solvents. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram immediately in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Weigh and powder 20 tablets. Add a quantity of the powder equivalent to about 0.1 g of Pyrazinamide, accurately weighed, to 200 ml of water, allow to stand for 10 minutes, swirling occasionally, mix in an ultrasonic bath for 10 minutes, and dilute to 500 ml with water. Filter and discard the first 20 ml of filtrate. Dilute 5 ml of the filtrate to 100 ml with water.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 268 nm. Calculate the content of $C_5H_5N_3O$, using the absorptivity value of 65.0 ($A^{1\%}_{1\,cm} = 650$).

QUININI DIHYDROCHLORIDI INJECTIO
QUININE DIHYDROCHLORIDE INJECTION

Description. Quinine dihydrochloride is an almost colourless to light yellow solution.

Category. Antimalarial drug.

Storage. Quinine dihydrochloride injection should be protected from light.

Labelling. The designation on the container should state that the solution must be diluted to a strength not exceeding 30 mg per ml before administration.

Additional information. Care must be taken to ensure slow intravenous administration.

Strength in the current WHO Model list of essential medicines: 300 mg/ml.

Requirements

Complies with the monograph for "Parenteral preparations".

Definition. Quinine dihydrochloride injection is usually a concentrated, sterile solution of quinine dihydrochloride in water for injections.

The solution is sterilized by "Heating in an autoclave" or any other suitable method (see 5.8 Methods of sterilization).

Quinine dihydrochloride injection contains not less than **95.0%** and not more than **105.0%** of the amount of $C_{20}H_{24}N_2O_2,2HCl$ stated on the label.

Identity tests

A. Dilute a volume of the injection to a concentration of 0.5 mg of Quinine dihydrochloride per ml. To 10 ml of this solution add 0.05 ml of sulfuric acid (~100 g/l) TS; a strong blue fluorescence is produced. (Keep the solution for test B.)

B. To the solution prepared for test A add 0.15 ml of bromine TS1 and 1 ml of ammonia (~100 g/l) TS; an emerald-green colour is produced.

C. The injection yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

pH value. pH of the injection, 1.5–3.0.

Related cinchona alkaloids. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 10 volumes of chloroform R, 8 volumes of acetone R, and 2.5 volumes of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of the following four solutions. For solution (A) dilute a volume of the injection with ethanol (~750 g/l) TS to obtain a concentration equivalent to 10 mg of Quinine dihydrochloride per ml. For solution (B) dissolve 12.5 mg of quinine R in 50 ml of ethanol (~750 g/l) TS. For solution (C) dissolve 12.5 mg of cinchonidine R in 50 ml of ethanol (~750 g/l) TS. For solution (D) mix 1 ml of solution B with 1 ml of solution C. After removing the plate from the chromatographic chamber, allow it to dry in a current of air for 15 minutes and repeat the development. Heat the plate at 105 °C for 30 minutes, allow to cool, spray with potassium iodoplatinate TS, and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B or solution C. If any spot is obtained

with solution A immediately below the principal spot, it is disregarded. The test is valid only if the chromatogram obtained with solution D shows two distinctly separated spots.

Limit of dihydroquinine. Dilute an accurately measured volume of the injection equivalent to 0.2 g of Quinine dihydrochloride with water to 20 ml. Add 0.5 g of potassium bromide R, 15 ml of hydrochloric acid (~70 g/l) TS, and 0.1 ml of methyl red/ethanol TS. Titrate with potassium bromate (0.0167 mol/l) VS until a yellow colour is produced. Add 0.5 g of potassium iodide R in 200 ml of water, stopper the flask, and allow to stand in the dark for 5 minutes. Titrate the iodine liberated by excess potassium bromate in the solution with sodium thiosulfate (0.1 mol/l) VS, adding 2 ml of starch TS when the solution has reached a light yellow colour. Repeat the procedure without the injection solution being examined and make any necessary corrections.

Each ml of potassium bromate (0.0167 mol/l) VS is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2,2HCl$. Express the results of both the above determination and the assay in percentages. The difference between the two is not more than 10%.

Assay. Transfer an accurately measured volume of the injection equivalent to about 0.5 g of Quinine dihydrochloride to a separator and add 20 ml of water and 5 ml of sodium hydroxide (~200 g/l) TS. Extract with successive quantities, each of 10 ml, of chloroform R until complete extraction of the alkaloid is effected. Wash each extract with the same two quantities, each of 5 ml, of water. Evaporate the combined chloroform layer, dissolve the residue in 50 ml of acetic anhydride R, add 10 ml of glacial acetic acid R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 19.87 mg of $C_{20}H_{24}N_2O_2,2HCl$.

STREPTOMYCINI SULFATIS PULVIS AD INJECTIONEM

STREPTOMYCIN SULFATE POWDER FOR INJECTIONS

Description. A white or almost white powder; odourless or with a slight odour.

Category. Antituberculosis drug.

Storage. Unless otherwise recommended by the manufacturer, the reconstituted suspension should be used within 4 days when stored at a temperature between 2 and 8 °C and protected from light.

Labelling. The designation on the container should state the dose in the equivalent amount of streptomycin. It should also state the nature of the buffering agent, preservatives, and stabilizers. Expiry date.

Additional information. Strength in the current WHO Model list of essential medicines: 1 g of streptomycin base.

Requirements

The powder for injections and the reconstituted solution for injections comply with the monograph for "Parenteral preparations".

Definition. Streptomycin sulfate powder for injections is a sterile powder of streptomycin sulfate.

Streptomycin sulfate powder for injections may contain suitable buffers, preservatives, and stabilizers. The powder is sterilized by a suitable method (see 5.8 Methods of sterilization).

Identity tests

- Either tests A and D or tests B, C, and D may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, but using the coating prepared as follows: to 0.3 g of carbomer R add 240 ml of water, mix, and shake moderately for 1 hour; then add gradually while stirring a sufficient volume of sodium hydroxide (~80 g/l) TS to adjust to pH 7.0. To this mixture add 30 g of silica gel R3 and coat the plate with a layer 0.75 mm thick. Heat the plate to 100 °C for 1 hour, cool, and use immediately. Use potassium dihydrogen phosphate (70 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following three solutions. For solution (A) dissolve a quantity of the powder for injections equivalent to 5 mg of Streptomycin sulfate in 5 ml of water. For solution (B) dissolve 10 mg of streptomycin sulfate RS in 10 ml of water. For solution (C) dissolve 1 mg of kanamycin monosulfate RS and 1 mg of framycetin sulfate RS in 1 ml of solution B. Develop the plate for a distance of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and spray with a mixture of equal volumes of naphthalene-1,3-diol/ethanol TS and sulfuric acid (~635 g/l) TS. Heat the plate to 150 °C for 5–10 minutes and examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows three clearly separated spots.

B. Dissolve a quantity of the powder for injections equivalent to 0.1 g of Streptomycin sulfate in 4 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS, and heat on a water-bath for 5 minutes. Cool and add 1.5 ml of hydrochloric acid (~70 g/l) TS and 1 drop of ferric chloride (25 g/l) TS; an intense violet colour is produced.

C. Dissolve a quantity of the powder for injections equivalent to 0.1 g of Streptomycin sulfate in 2 ml of water, add 1 ml of 1-naphthol TS1 and 2 ml of a mixture of equal volumes of sodium hypochlorite (~40 g/l) TS and water; a red colour is produced.

D. A solution containing a quantity of the powder for injections equivalent to 50 mg of Streptomycin sulfate yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Clarity and colour of solution. A solution of the powder for injections equivalent to 2.5 g of Streptomycin sulfate in 10 ml of carbon-dioxide-free water R is clear and colourless. (Keep this solution for the "pH value".)

Loss on drying. Dry the powder for injections at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours; it loses not more than 70 mg/g.

pH value. pH of the solution prepared above for the test of clarity and colour, 5.0–8.0.

Assays

Mix the contents of 10 containers and carry out the assays as described.

Potency. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (a) *Bacillus subtilis* (NCTC 8236, or ATCC 11774) as the test organism, culture medium Cm1 with a final pH of 7.9–8.0, sterile phosphate buffer, pH 8.0, TS1 or TS2, an appropriate concentration of streptomycin (usually between 5 and 20 IU/ml), and an incubation temperature of 36–39 °C, or (b) *Bacillus subtilis* (ATCC 6633) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS1 or TS2, an appropriate concentration of streptomycin (usually between 3 and 15 IU/ml) and an incubation temperature of 35–37 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency.

The upper fiducial limit of error is not less than 720 IU per mg calculated with reference to the dried substance. Using the average contents and estimated potency, calculate the total number of units of streptomycin in the container.

In places where the microbiological determination is not feasible perform the following alternative assay.

Streptomycin sulfate. Dissolve a quantity of the powder for injections equivalent to about 0.1 g of Streptomycin sulfate, accurately weighed, in sufficient water to produce 100 ml. To 5 ml add 5 ml of sodium hydroxide (0.2 mol/l) VS and heat in a water-bath for exactly 10 minutes. Cool in ice for exactly 5 minutes. Add 3 ml of ferric ammonium sulfate TS2, as well as sufficient water to produce 25 ml, mix, and allow to stand for exactly 20 minutes after the addition of the last reagent.

Immediately measure the absorbance of a 1-cm layer at the maximum at about 525 nm, against a solvent cell containing a solution prepared in the same manner but omitting the powder for injections being examined. Calculate the content of $(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$ in the powder for injections, using the absorptivity value of 1.18 ($A^{1\%}_{1\,cm} = 11.8$). Using the result and the average mass of contents, calculate the total content of streptomycin in the container, using the conversion 800 mg of streptomycin being equivalent to 1 g of Streptomycin sulfate. The container of Streptomycin sulfate powder for injections contains not less than 90.0% and not more than 115.0% of the amount of $C_{21}H_{39}N_7O_{12}$ stated on the label.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.25 IU of endotoxin RS per mg of streptomycin.

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure.

Monographs

Radiopharmaceuticals

RADIOPHARMACEUTICALS

The handling and testing of radiopharmaceuticals (radioactive pharmaceuticals) require specialized techniques in order that correct results may be obtained and hazards to personnel minimized. All operations should be carried out or supervised by personnel who have received expert training in handling radioactive materials.

Definitions

Nuclide

A species of atom characterized by its mass number, atomic number, and nuclear energy state, provided that the mean life in that state is long enough to be observable.

Radioactivity

The property of certain nuclides of emitting radiation by the spontaneous transformation of their nuclei into those of other nuclides.

EXPLANATORY NOTE. The term "disintegration" is widely used as an alternative to the term "transformation". Transformation is preferred as it includes, without semantic difficulties, those processes in which no particles are emitted from the nucleus.

Radionuclide

A nuclide that is radioactive.

Units of radioactivity

The activity of a quantity of radioactive material is expressed in terms of the number of nuclear transformations taking place in unit time. The SI unit of activity is the becquerel (Bq), a special name for the reciprocal second (s^{-1}). The expression of activity in terms of the becquerel therefore indicates the number of disintegrations per second. One curie (Ci) equals 3.7×10^{10} Bq.

The conversion factors between becquerel and curie and its submultiples are given in "Units of measurement".

Half-life period

The time in which the radioactivity decreases to one-half its original value.

EXPLANATORY NOTE. The rate of radioactive decay is constant and characteristic for each individual radionuclide. The exponential decay curve is described mathematically by the equation

$$N = N_o e^{-\lambda t}$$

where N is the number of atoms at elapsed time t, N_0 is the number of atoms when $t = 0$, and λ is the disintegration constant characteristic of each individual radionuclide. The half-life period is related to the disintegration constant by the equation

$$T_{\frac{1}{2}} = \frac{0.693}{\lambda}$$

Radioactive decay corrections are calculated from the exponential equation, or from decay tables, or are obtained from a decay curve plotted for the particular radionuclide involved (see Fig. 1).

Radioactive concentration

The radioactive concentration of a solution refers to the radioactivity in a unit volume of the solution. As with all statements involving radioactivity, it is necessary to include a reference date of standardization. For radionuclides with a half-life period of less than 30 days, the time of standardization should be expressed to the nearest hour. For radionuclides with a half-life period of less than one day, a more precise statement of the reference time is required.

Specific radioactivity (or specific activity)

The specific activity of a preparation of a radioactive material is the radioactivity per unit mass of the element or of the compound concerned.

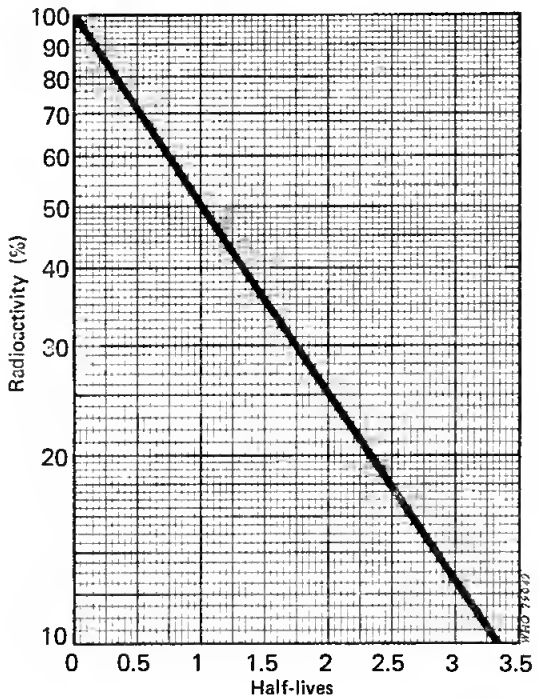


Figure 1. Master Decay Chart.

EXPLANATORY NOTE. It is usual to specify the radionuclide concerned and also it is necessary to express the time thus: "1 mCi of iodine-131 per mg of *o*-iodohippuric acid at 12.00 hours UT on 1 January 1979", or "40 MBq of selenium-75 per mg of selenomethionine on 1 January 1979".

Specific radioactivity is often not determined directly but is calculated from a knowledge of the radioactive concentration of the solution and of the chemical concentration of the radioactive compound. Thus, if a solution contains *x* mCi of ^{131}I per ml, and if the ^{131}I is entirely in the chemical form of sodium *o*-iodohippurate of which the concentration is *y* mg per ml, then at that time the specific activity is:

x/*y* mCi of iodine-131 per mg of *o*-iodohippuric acid.

Where necessary, the radiochemical purity of the preparation (see below) must be taken into account.

The term employed in radiochemical work is "specific activity". As the word "activity" has other connotations in a pharmacopoeia, the term should, where necessary, be modified to "specific radioactivity" to avoid ambiguity.

Radionuclidic purity

The radionuclidic purity of a preparation is that percentage of the total radioactivity that is present in the form of the stated radionuclide.

EXPLANATORY NOTE. Some radionuclides decay into nuclides that are themselves radioactive: these are referred to as mother (or parent) and daughter radionuclides, respectively. Such daughter radionuclides are often excluded when calculating the radionuclidic purity; for example, iodine-131 will always contain its daughter xenon-131m, but this would not be considered an impurity because its presence is unavoidable.

In employing the definition, the radioactivity must be measured in appropriate units: that is, in the number of nuclear transformations that occur in unit time (in terms of curies or becquerels). If, for example, a preparation stated to be iodine-125 is known to contain 99 mCi of iodine-125 and 1 mCi of iodine-126, and no other radionuclide, then the preparation is said to be of 99% radionuclidic purity. It will be noted that the relative amounts of iodine-125 and iodine-126, and hence the radionuclidic purity, will change with time. An expression of radionuclidic purity must therefore contain a statement of the time, such as: "Not more than 1% of the total radioactivity is due to iodine-126 at the reference date stated on the label". In the case of radionuclides with a half-life period of less than 30 days the reference hour should also be included.

It is clear that, in order to give a statement of the radionuclidic purity of a preparation, the activities (and hence the identities) of every radionuclide present must be known. There are no simple and certain means of identifying and measuring all the radionuclidic impurities that might be present in a preparation. An expression of radionuclidic purity must either depend

upon the judgement of the person concerned, or it must be qualified by reference to the method employed, for example: "No radionuclidic impurities were detected by gamma scintillation spectrometry using a sodium iodide detector."

Radiochemical purity

The radiochemical purity of a preparation is that percentage of the stated radionuclide that is present in the stated chemical form. As radiochemical purity may change with time, mainly because of radiation decomposition, the time at which the radiochemical purity limit is applicable must be specified.

EXPLANATORY NOTE. If, for example, a preparation of cyanocobalamin (^{57}Co) is stated to be 99% radiochemically pure, then 99% of the cobalt-57 is present in the form of cyanocobalamin. Radiochemical impurities might include such substances as cobaltous (^{57}Co) ion or hydroxycobalamin (^{57}Co).

The possible presence of radionuclidic impurities is not taken into account in the definition. If the radionuclidic impurity is not isotopic with the stated radionuclide, then it cannot possibly be in the identical chemical form. If the radionuclidic impurity is isotopic with the stated radionuclide, it could be, and indeed is likely to be, in the same chemical form.

Radiochemical impurities may arise during the preparation of the material or during storage, because of ordinary chemical decomposition or, what is often more important, because of radiation decomposition (that is, because of the physical and chemical effects of the radiation).

Production and handling of radiopharmaceuticals

The following paragraphs concern special considerations applying to the monographs on radiopharmaceuticals. The facilities for the production, use, and storage of radiopharmaceuticals are generally subject to licensing by national authorities. They often have to comply with 2 sets of regulations, those concerned with pharmaceutical preparations and those concerned with radioactive materials. Each producer or user must be thoroughly cognizant of the national requirements pertaining to the articles concerned.

Carriers

The mass of radioactive material usually encountered in radioactive pharmaceuticals is often too small to be measured by ordinary chemical or physical methods. Since such small amounts may not be subject to the usual methods of separation and purification, a *carrier*, in the form of inactive material, either isotopic with the radionuclide, or non-isotopic, but chemically similar to the radionuclide, may be added during processing and dispensing to permit ready handling. Thus sodium phosphate carrier is present in "Natrii Phosphatis (^{32}P) Injectio" and rhenium is used as a carrier in certain colloidal preparations of technetium-99m. The amount of carrier added must be sufficiently small for it

not to cause undesirable physiological effects. The mass of an element formed in a nuclear reaction may be exceeded by that of the inactive isotope present in the target material or in the reagents used in the separation procedures.

Radioactive preparations in which no carrier is intentionally added during the manufacture or processing are often loosely referred to as *carrier-free*.

Detection and measurement

Radioactive transformations may involve the emission of charged particles, the process of electron capture, or the process of isomeric transition. The charged particles emitted from the nucleus may be alpha particles (helium nuclei of mass number 4) or beta particles (electrons of negative or positive charge, $\beta-$ or $\beta+$ respectively, the latter known as positrons). The emission of charged particles from the nucleus may be accompanied by gamma rays, which are of the same physical nature as X-rays. Gamma rays are also emitted in the process of isomeric transition (i.t.). X-rays, which may be accompanied by gamma rays, are emitted in the process of electron capture (e.c.). Positrons are annihilated on contact with matter. Each positron annihilated is accompanied by the emission of 2 gamma rays, each with an energy of 0.511 MeV.

The physical characteristics of radionuclides are summarized in Table 1.

The methods employed for the detection and measurement of radioactivity are dependent upon the nature and energy of the radiation emitted. Radioactivity may be detected and/or measured by a number of different instruments based upon the action of radiation in causing the ionization of gases and solids, or the fluorescence of certain solids and liquids, or by the effect of radiation on a photographic emulsion.

In general, a counting assembly consists of a sensing unit and an electronic scaling device. The sensing unit may be a Geiger-Müller tube, a proportional counter, a scintillation detector in which a photomultiplier tube is employed in conjunction with a scintillator, or a solid-state semiconductor.

Geiger-Müller counters and proportional counters are generally used for the measurement of the beta emitters. Scintillation counters employing liquid or solid phosphors may be used for the measurement of alpha, beta, and gamma emitters. Solid-state devices may also be used for alpha, beta, and gamma measurements. The electronic circuitry associated with a detector system usually consists of a high-voltage supply, an amplifier, a pulse-height selector, and a sealer, a ratemeter, or other readout device. When the electronic scaling device or the sealer in a counting assembly is replaced by an electronic integrating device, the resultant assembly is a ratemeter. Ratemeters are used for the purpose of monitoring and surveying radioactivity and are somewhat less precise as measuring instruments than the counters. Ionization chambers are often used for measuring gamma-ray activities and, provided they are thin-walled, for measuring X-rays.

Radiation from a radioactive source is emitted in all directions. Procedures for the standardization and measurement of such sources by means of a count of the emissions in all directions are known as 4π-counting; those based on

Table 1. Physical characteristics of radionuclides

Nuclide	Half-life period[a]	Type of decay[b]	Particle energies and transition probabilities		Electromagnetic transitions		
			energy MeV	transition probability	photon energy MeV	photons emitted	transitions internally converted
Cesium-137	30.1 a	β^-	0.512	94.6%			
			1.174	5.4%			
					via 2.6 min ^{137m}Ba		
					0.662	85.1%	9.5%
					0.032–0.038	8% (Ba K X-rays)	
Chromium-51	27.7 d	e.c.		100%	0.320	9.83%	
					0.005–0.006	~22% (V K X-rays)	
Cobalt-57	270 d	e.c.		100%	0.014	9.4%	78.0%
					0.122	85.2%	2.0%
					0.136	11.1%	1.5%
					0.570	0.02%	
					0.692	0.16%	
					others	low intensity	
					0.006–0.007	~55% (Fe K X-rays)	
Cobalt-58	70.8 d	β^+	0.475	15.0%	0.511	from β^+	
		e.c.		85.0%	0.811	99.4%	
					0.864	0.7%	
					1.675	0.5%	
					0.006–0.007	~26% (Fe K X-rays)	
Cobalt-60	5.27 a	β^-	0.318	99.9%	1.173	99.86%	0.02%
			1.491	0.1%	1.333	99.98%	0.01%
					others	<0.01%	

Gallium-67	78.3 h	e.c.		100%	0.091	3.6%	0.3%
					0.185	23.5%	0.4%
					0.209	2.6%	0.02%
					0.300	16.7%	0.06%
					0.394	4.4%	0.01%
					0.494	0.1%	
					0.704	0.02%	
					0.795	0.06%	
					0.888	0.17%	
					0.008–0.010	43% (Zn K X-rays)	
					via 9.2 μs ^{67m}Zn		
					0.093	37.6%	32.4%
					0.008–0.010	13% (Zn K X-rays)	
Gold-198	2.70 d	β^-	0.285	1.32%	0.412	95.45%	4.3%
			0.961	98.66%	0.676	1.06%	0.03%
			1.373	0.02%	1.088	0.23%	
Gold-199	3.13 d	β^-	0.25	21%	0.050	0.3%	3.5%
			0.29	72%	0.158	39.6%	36.4%
			0.45	7%	0.208	8.8%	8.3%
					0.069–0.083	~18% (Hg K X-rays)	
Indium-111	2.81 d	e.c.		100%	0.172	89.6%	10.4%
					0.247	94.0%	6.0%
Indium-113m	99.5 min	i.t.		100%	0.392	64.9%	35.1%
					0.024–0.028	24% (In K X-rays)	
Iodine-123	13.2 h	e.c.		100%	0.159	83.0%	16.3%
					0.347	0.10%	
					0.440	0.35%	
					0.506	0.26%	
					0.529	1.05%	
					0.539	0.27%	
					0.027–0.032	~86% (Te K X-rays)	

Table 1. Continued

Nuclide	Half-life period[a]	Type of decay[b]	Particle energies and transition probabilities		Electromagnetic transitions		
			energy MeV	transition probability	photon energy MeV	photons emitted	transitions internally converted
Iodine-125	60.0 d	e.c.		100%	0.035	7%	93%
					0.027–0.032	138% (Te K X-rays)	
Iodine-126	13 d	β^-	0.38	3%	0.389	32%	0.5%
			0.88	30%	0.491	2%	
			1.27	15%	0.511	from β^+	
		β^+	0.46	~0.1%	0.666	30%	0.1%
			1.1	~0.4%	0.754	4%	
		e.c.		51.5%	0.880	0.8%	
					1.420	0.3%	
					others	<0.1% each	
					0.027–0.032	~38% (Te K X-rays)	
Iodine-131	8.06 d	β^-	0.247	1.8%	0.080	2.4%	3.8%
			0.304	0.6%	0.284	5.9%	0.3%
			0.334	7.2%	0.364	81.8%	1.7%
			0.606	89.7%	0.637	7.2%	
			0.806	0.7%	0.723	1.8%	
		1.3% of ^{131}I decays via 12 d ^{131m}Xe					
(Xenon-131m)		i.t.		100%	0.164	2%	98%
			(percentages relate to disintegrations of ^{131m}Xe)				
Iodine-132	2.29 h	β^-	0.84	16.0%	0.506	5.0%	
			1.01	3.5%	0.523	16.1%	0.2%
			1.07	6.5%	0.621	2.0%	
			1.09	3.0%	0.630	13.7%	0.1%

			1.10	2.6%	0.651	2.7%	
			1.26	2.9%	0.668	98.7%	0.4%
			1.29	18.4%	0.670	4.9%	
			1.57	10.8%	0.672	5.2%	
			1.72	12.7%	0.727	6.5%	
			2.24	20.2%	0.773	76.2%	0.3%
			others	3.4%	0.810	2.9%	
					0.812	5.6%	
					0.955	18.1%	
					1.136	3.0%	
					1.295	2.0%	
					1.372	2.5%	
					1.399	7.1%	
					1.433	1.4%	
					1.921	1.2%	
					2.002	1.1%	
					others	<1.5%	
Iron-55	2.69 a	e.c.			0.006	~28% (Mn K X-rays)	
Iron-59	44.6 d	β^-	0.084	0.1%	0.143	0.8%	
			0.132	1.1%	0.192	2.8%	
			0.274	45.8%	0.335	0.3%	
			0.467	52.7%	0.383	0.02%	
			1.566	0.3%	1.099	55.8%	
					1.292	43.8%	
					1.482	0.06%	
Mercury-197	64.4 h	e.c.		100%	0.077	19.2%	80.7%
					0.192	~1.1%	0.9%
					0.268	~0.1%	
					0.067–0.080	~73% (Au K X-rays)	

Table 1. Continued

Nuclide	Half-life period[a]	Type of decay[b]	Particle energies and transition probabilities		Electromagnetic transitions		
			energy MeV	transition probability	photon energy MeV	photons emitted	transitions internally converted
Mercury-197m	24 h	e.c.		6.5%	0.134	31.8%	61.7%
		i.t.		93.5%	0.165	0.3%	93.2%
					0.067–0.083	36% (Au/Hg K X-rays)	
					via 7.8 s ^{197m}Au		
					0.130	0.5%	6%
					0.279	5.0%	1.5%
					0.409	<0.005%	
					0.067–0.080	~2% (Au K X-rays)	
		Daughter ^{197}Hg					
Mercury-203	46.6 d	β^-	0.212	100%	0.279	81.5%	18.5%
					0.071–0.085	12.8% (Tl K X-rays)	
Molybdenum-99	66.2 h	β^-	0.454	18.3%	0.041	1.2%	4.8%
			0.866	1.4%	0.141	5.4%	0.7%
			1.232	80%	0.181	6.6%	1.0%
			others	0.3%	0.366	1.4%	
					0.412	0.02%	
					0.529	0.05%	
					0.621	0.02%	
					0.740	13.6%	
					0.778	4.7%	
					0.823	0.13%	
					0.961	0.1%	
					via 6.02 h ^{99m}Tc in equilibrium		
					0.002	~0%	93.9%

					0.141	83.9%	10.0%
					0.143	0.03%	0.8%
Phosphorus-32	14.3 d	β^-	1.709	100%			
Selenium-75	118.5 d	e.c.		100%	0.066	1.1%	0.3%
					0.097	2.9%	3.0%
					0.121	15.7%	0.7%
					0.136	54.0%	1.6%
					0.199	1.5%	
					0.265	56.9%	0.4%
					0.280	18.5%	0.2%
					0.401	11.7%	
					others	<0.05% each	
					0.010–0.012	~50% (As K X-rays)	
					via 16.4 ms ^{75m}As		
					0.024	0.03%	5.5%
					0.280	5.4%	
					0.304	1.2%	0.1%
					0.010–0.012	~2.6% (As K X-rays)	
Technetium-99m	6.02 h	i.t.		100%	0.002	~0%	99.1%
					0.141	88.5%	10.6%
					0.143	0.03%	0.87%
		Daughter ^{99}Tc					
Thallium-201	73.5 h	e.c.		100%	0.031	0.29%	10.1%
					0.032	0.25%	9.6%
					0.135	2.9%	8.9%
					0.166	0.13%	0.2%
					0.167	8.81%	16.0%
Tin-113	115 d	e.c.		100%	0.255	21%	0.1%
					0.024–0.028	73% (In K X-rays)	
		Daughter ^{131m}In					

Table 1. Continued

Nuclide	Half-life period[a]	Type of decay[b]	Particle energies and transition probabilities		Electromagnetic transitions		
			energy MeV	transition probability	photon energy MeV	photons emitted	transitions internally converted
Tritium (^{3}H)	12.35 a	β^-	0.0186	100%			
Xenon-131m	11.9 d	i.t.		100%	0.164	2%	98%
					0.029–0.035	~52% (Xe K X-rays)	
Xenon-133	5.25 d	β^-	0.266	0.9%	0.080	0.4%	0.5%
			0.346	99.1%	0.081	36.6%	63.3%
					0.160	0.05%	
					0.030–0.036	~46% (Cs K X-rays)	
Xenon-133m	2.26 d	i.t.		100%	0.233	8%	92%
					0.029–0.035	~59% (Xe K X-rays)	
		Daughter ^{133}Xe					
Ytterbium-169	32.0 d	e.c.		100%	0.021	0.21%	12.3%
					0.063	45.16%	50.4%
					0.094	0.78%	12.3%
					0.110	3.82%	56.2%
					0.117	0.04%	
					0.118	1.90%	3.2%
					0.131	11.42%	13.5%
					0.177	17.31%	17.7%
					0.198	26.16%	25.7%
					0.240	0.12%	
					0.261	1.74%	
					0.308	11.04%	0.7%

[a] µs = microsecond; ms = millisecond; s = second; min = minute; h = hour; d = day; a = year.

[b] e.c. = electron capture; i.t. = isomeric transition.

a count of the emissions in a solid angle of 2π steradians are known as 2π-counting; and those based on a fraction of the emissions defined by the solid angle subtended from the detector to the source are known as counting in a fixed geometry. It is customary to assay the radioactivity of a preparation by comparison with a standardized preparation using identical geometry conditions. The validity of such an assay is critically dependent upon the reproducibility of the spatial relationships of the source to the detector and its surroundings and upon the accuracy of the standardized preparation. In the primary standardization of radionuclides coincidence techniques are employed in preference to simple 4π-counting whenever the decay scheme of the radionuclide permits. One of the most commonly employed coincidence techniques is 4π-beta/gamma coincidence counting, which is used for nuclides in which some or all of the disintegrations are followed by prompt photon emission. An additional adjacent detector, sensitive only to photons, is used to measure the efficiency in the 4π-counter of those disintegrations with which the photons are coincident. 4π-Gamma/gamma coincidence counting techniques are often employed for the standardization of pure gamma emitters.

The construction and performance of instruments and accessory apparatus vary. The preparation of samples must be modified to obtain satisfactory results with a particular instrument. The operator must follow carefully the manufacturer's instructions for optimum instrument performance and substantiate results by careful examination of known samples. Proper instrument functioning and reliability must be monitored on a day-to-day basis through the use of secondary reference preparations.

Radioactivity due to materials of construction, to cosmic rays, and to spontaneous discharges in the atmosphere contributes what is known as the *background activity*. All sample radioactivity measurements must be corrected by subtracting background activity.

In the counting of samples at high activity levels, corrections must be made also for loss of counts due to inability of the equipment to resolve pulses arriving in close succession. Such coincidence-loss corrections must be made prior to the subtraction of background correction.

The corrected count rate, R, is given by the formula

$$R = \frac{r}{1 - r\tau}$$

where r is the observed count rate, and τ is the resolving time.

A radioactivity count is a statistical value, i.e. it is a measure of nuclear decay probabilities, and is not exactly constant over any given time interval. The magnitude of the standard deviation is approximately equal to the square root of the number of counts. In general, at least 10000 counts are necessary to obtain a standard deviation of 1%.

Absorption

Ionizing radiation is absorbed in the material surrounding the source of the radiation. Such absorption occurs in air, in the sample itself (self-absorption), in sample coverings, in the window of the detection device, and in any special absorbers placed between the sample and the detector. Since alpha particles have a short range of penetration in matter, beta particles have a somewhat greater range, and gamma rays are deeply penetrating, identification of the type and energy of radiation emitted from a particular radionuclide may be determined by the use of absorbers of varying thickness. In practice, this method is little used, and then only in connection with beta emitters. However, variations in counting rate due to variations in thickness and density of sample containers can be a major problem with beta emitters and with X-ray emitters, such as iodine-125. Plastic tubes, in which variations of density and thickness are minimal, are, therefore, often employed.

The absorption coefficient (μ), which is the reciprocal of the "thickness" expressed in mg/cm^2, or the half-thickness (the thickness of absorber required to reduce the radioactivity by a factor of two), is commonly determined to characterize the beta radiation emitted by a radionuclide.

Method

The following procedure is used for the identification test in "Natrii Phosphatis (^{32}P) Injectio" for the measurement of beta activity and for calculation of the absorption coefficient of half-thickness:

Place the radioactive substance, suitably mounted for counting, under a suitable counter. Make count rate determinations individually and successively, using at least 6 different thicknesses of aluminium foil chosen from a range of 10 to 200 mg/cm^2 and a single absorber with a thickness of at least 800 mg/cm^2. The sample and absorbers should be as close as possible to the detector in order to minimize scattering effects. Obtain the net beta count rate at the various absorbers used by subtracting the count rate found with the thickest absorber (800 mg/cm^2 or more). Plot the logarithm of the net beta count rate as a function of the total absorber thickness. The total absorber thickness is the thickness of the aluminium plus the thickness of the counter window (as stated by the manufacturer), plus the air-equivalent thickness (the distance, expressed in cm, of the sample from the counter window multiplied by 1.205), all expressed in mg/cm^2. An approximately straight line results.

Choose two of the absorber thicknesses (t_1 and t_2) that are 20 mg/cm^2 or more apart and that fall on the plot, and calculate the absorption coefficient (μ) from the equation

$$\mu = \frac{1}{t_2 - t_1} \ln \frac{A_{t_1}}{A_{t_2}}$$

where t_1 is the thinner absorber, t_2 is the thicker absorber, and A_{t_1} and A_{t_2} represent the net beta count rate with t_1 and t_2 absorbers, respectively. Alternatively the half-thickness may be read directly from the plot.

The choice of absorber thickness depends on the radionuclide. For radionuclides other than phosphorus-32, which have higher or lower beta energy, greater or lesser absorber thicknesses are necessary.

For characterization of the radionuclide, the absorption coefficient or the half-thickness should be within ±5% of that found for a sample of the same radionuclide of known purity when determined in parallel.

The count rate at zero total absorber thickness may be determined by plotting a curve identical with the one described for determination of the absorption coefficient and extrapolating the straight line plot to zero absorber thickness, taking into consideration the thickness, expressed in mg/cm^2, of sample coverings, the air, and the counter window.

Radiation spectrometry

Crystal scintillation spectrometry

When the energy of beta or gamma radiation is dissipated within materials known as scintillators, light is produced in an amount proportional to the energy dissipated. This quantity of light may be measured by suitable means, and is proportional to the energy absorbed in the scintillator. The light emitted under the impact of a gamma photon or a beta particle is converted into an electric output pulse by a photomultiplier. Scanning of the output pulses with a suitable pulse-height analyser results in an energy spectrum of the source.

The scintillators most commonly used for gamma spectrometry are single crystals of thallium-activated sodium iodide. Gamma-ray scintillation spectra show one or more sharp, characteristic photoelectric peaks, corresponding to the energies of the gamma radiation of the source. They are thus useful for identification purposes and also for the detection of gamma-emitting impurities in a preparation. These peaks are accompanied by other peaks due to secondary effects of radiation on the scintillator and its surroundings, such as backscatter, positron annihilation, coincidence summing, and fluorescent X-rays. In addition, broad bands known as the Compton continua arise from the scattering of the gamma photons in the scintillator and in surrounding materials. Calibration of the instrument is achieved with the use of known samples of radionuclides whose energy spectra have been characterized. The shape of the spectrum produced will vary with the instrument used, owing to such factors as differences in the shape and size of the crystal, in the shielding materials used, the distance between the source and the detector, and in the types of discriminator employed in the pulse-height analysers. When using the spectrum for identification of radionuclides it is, therefore, necessary to compare the spectrum with that of a known sample of the radionuclide obtained in the same instrument under identical conditions.

Certain radionuclides, for example, iodine-125, emit characteristic X-rays of well-defined energies that will produce photoelectric peaks in a suitable gamma spectrometer. Beta radiation also interacts with the scintillators, but the spectra are continuous and diffuse and generally of no use for identification of the radionuclide or for the detection of beta-emitting impurities in a preparation.

Semiconductor detector spectrometry

Gamma-ray and beta-particle spectra may be obtained using solid-state detectors. The peaks obtained do not suffer to the same extent the broadening shown in crystal scintillation spectrometry, and the resolution of gamma photons of similar energies is very much improved. However, the efficiencies of such detectors are much lower.

The energy required to create an electron-hole pair or to promote an electron from the valence band to the conduction band in a semiconductor is far less than the energy required to produce a photon in a scintillation crystal. In gamma-ray spectrometry a lithium-drifted germanium detector can provide an energy resolution of 0.33% for the 1.33 MeV photon of cobalt-60, as compared with 5.9% with a 7.6-cm × 7.6-cm thallium-activated sodium iodide crystal.

Liquid scintillation counting

For weak beta emitters like ^{35}S, ^{14}C and ^{3}H, where self-absorption of the low-energy beta particles is significant, the preferred counting method is by liquid scintillation, which can occasionally be employed also for emitters of X-rays, alpha particles, and gamma-rays. If the sample to be counted is dissolved in, or mixed with, a solution of an appropriate scintillator material, the decay energy from the sample is converted into light photons. These are sensed by a photomultiplier, which converts them into an electric pulse, whose intensity is proportional to the energy of the initial radiation. Thus, simultaneous counting of several radionuclides differing in the energy of emitted radiation can be effected with suitable discriminators (pulse-height analysers), providing the energy separation is sufficient. Detection efficiencies approaching 95% for ^{14}C and 60% for ^{3}H are reached because self-absorption is minimized.

The scintillator solute usually consists of a polycyclic aromatic compound, such as *p*-terphenyl or 2,5-diphenyloxazole (primary solute), together with a secondary solute, such as 1,4-di[2-(4-methyl-5-phenyloxazole)]benzene (Dimethyl-POPOP), that shifts the wavelength of the light emitted to match the highest sensitivity of the photomultiplier tube. Water-immiscible solvents, such as toluene, or water-miscible solvents, such as dioxan, can be used. To facilitate the counting of aqueous solutions, special solvents have been developed. Alternatively, samples may be counted as suspensions in scintillator gels. As a means of attaining compatibility and miscibility with aqueous specimens to be assayed, many additives, such as surfactants and solubilizing agents, are also incorporated into the scintillator. For accurate determination of sample radioactivity, care must be exercised to prepare a sample that is truly homogeneous. The presence of impurities and colour in the solution causes a decrease in the number and energy of photons reaching the photomultiplier tube; such a decrease is known as quenching. Accurate radioactivity measurement requires correcting for count-rate loss due to quenching. Solutions containing organic scintillators are prone to photoexcitation and samples may need to be prepared in subdued light and kept in darkness before counting.

Radiation shielding

Adequate shielding must be used to protect laboratory personnel from ionizing radiation, and measuring instruments must be suitably shielded from background radiation.

Alpha and beta radiations are readily shielded because of their limited range of penetration, although the production of *Bremsstrahlung* by the latter must be taken into account (see below). The range of alpha and beta particles varies inherently with their kinetic energy. The alpha particles are monoenergetic and have a range of a few centimetres in air. The absorption of beta particles, owing to their continuous energy spectrum and scattering, follows an approximately exponential function. The range of beta particles in air varies from centimetres to metres.

The secondary radiation produced by beta radiation upon absorption by shielding materials is known as *Bremsstrahlung* and resembles soft X-rays in its property of penetration. The higher the atomic number or density of the absorbing material, the greater the intensity of the *Bremsstrahlung* produced. Elements of low atomic number produce low-energy *Bremsstrahlung,* which is readily absorbed; therefore, materials of low atomic number or of low density, such as aluminium, glass, or transparent plastic, are used to shield sources of beta radiation.

Gamma-ray radiation is deeply penetrating. Attenuation of gamma-ray radiation in matter is exponential and is given in terms of half-value layers. The *half-value layer* is the thickness of shielding material necessary to decrease the intensity of radiation to half its initial value. A shield of 7 half-value layers is of a thickness that will reduce the intensity of radiation to less than 1% of its unshielded intensity of activity. Gamma-ray radiation is commonly shielded with lead.

Intensity of gamma-ray radiation is diminished according to the inverse square of the intervening distance between the source and the point of reference. Radioactive materials of multimillicurie strength can be handled safely in the laboratory by using proper shielding and/or by arranging for the maximum practicable distance between the source and the operator by means of remote-handling devices.

Determination of radionuclidic purity

For gamma emitters the most useful method of examination for radionuclidic purity is gamma spectrometry. It is not, however, a completely certain method, because:

(*a*) beta-emitting impurities are, in general, not detected;
(*b*) when sodium iodide detectors are employed, the photoelectric peaks due to impurities may be obscured by those due to the major radionuclide, or, in other words, the degree of resolution of the instrument is insufficient; this problem can be overcome through the use of high-resolution,

solid-state, semiconductor detectors, such as a lithium-drifted germanium (Ge:Li) detector;

(*c*) unless the instrument has been calibrated with a standard source of known radionuclidic purity under identical conditions of geometry, it is difficult to determine whether additional peaks are due to impurities or whether they result from such secondary effects as backscatter, coincidence summation, or fluorescent X-rays.

The range of gamma spectrometry may be extended in two ways: first, by observing changes in the spectrum of a preparation with time (this is especially useful in detecting the presence of long-lived impurities in a preparation of a short-lived radionuclide); secondly, by the use of chemical separations, whereby the major radionuclide may be removed by chemical means and the residue examined for impurities, or whereby specific impurities may be separated chemically and then quantified. It is evident that chemical means will not separate an impurity that is isotopic with the major radionuclide.

Requirements for radionuclidic purity

Requirements for radionuclidic purity are specified in two ways:

1. By expression of a minimum level of radionuclidic purity. Unless otherwise stated in the individual monograph, the gamma-ray spectrum, as determined by simple gamma spectrometry employing a sodium iodide detector, should not be significantly different from that of a standardized solution of the radionuclide before the expiry date is reached. As discussed above, it is difficult to set more precise requirements for a minimum level of radionuclidic purity.
2. By expression of maximum levels of specific radionuclidic impurities in the individual monographs. In general, such impurities are those that are known to be likely to arise during the production of the material – for example, mercury-203 in a preparation of mercury-197.

It is evident that while the above requirements are necessary, they are not in themselves sufficient to ensure that the radionuclidic purity of a preparation is sufficient for human use. A duty must remain with the manufacturer to examine his products in detail, and especially to examine preparations of short-lived radionuclides for long-lived impurities after a suitable period of decay. In this way, the manufacturer may satisfy himself that the manufacturing processes employed are producing materials of appropriate purity. In particular, the radionuclidic composition of certain preparations is determined by the chemical and isotopic composition of the target material, which is irradiated with neutrons, and trial preparations are advisable when new batches of target material are employed.

Determination of radiochemical purity

Radiochemical purity can be studied by a variety of techniques, but 1.14.2 Paper chromatography and 1.14.1 Thin-layer chromatography are of particular importance. After completion of the separation, the distribution of radioactivity on the chromatogram is determined. The weight of substance applied to the chromatogram is often extremely small (because of the great sensitivity of detection of the radioactivity) and particular care has to be taken in interpretation with regard to the formation of artefacts. Instead of chromatography, electrophoresis may be used for separation (see 1.15 Electrophoresis). As mentioned above, the addition of carriers (i.e. the corresponding non-radioactive compounds) for both the radiopharmaceutical itself and the suspected impurities is sometimes helpful. There is, however, a danger that when an inactive carrier of the radiopharmaceutical is added it may interact with the radiochemical impurity, leading to underestimation of these impurities. Another useful technique involves monitoring the biological distribution of the injected radiopharmaceutical in suitable test animals.

Determination of chemical purity

Chemical purity refers to the proportion of the preparation that is in the specified chemical form regardless of the presence of radioactivity; it may be determined by normal methods of analysis.

The chemical purity of a preparation is often no guide to its radiochemical purity. Preparations, especially those resulting from exchange reactions (for example, a preparation of *o*-iodohippuric acid in which some of the iodine atoms are replaced by atoms of iodine-131), may be of high chemical purity but may contain impurities of high specific activity (that is, a tiny weight of an impurity may be associated with a relatively large amount of the radionuclide).

In general, chemical impurities in preparations of radiopharmaceuticals are objectionable only if they are toxic or if they modify the physiological processes that are under study.

Tests for sterility and pyrogens

A number of monographs for radiopharmaceuticals contain the requirement that the product be sterile and free of pyrogens. The half-life of radiopharmaceutical products is such that, as a rule only tests for pyrogens can be completed prior to release. Tests for sterility must, in general, be completed retrospectively.

Sterility tests

The manufacturer should begin the sterility test as soon as possible and read the results after release.

A particular responsibility falls upon the manufacturer of radiopharmaceuticals to validate the sterilization process by all suitable measures, which may

include careful and frequent calibration of sterilizers and the use of biological and chemical indicators of the efficiency of the sterilization process.

Pyrogen tests

The manufacturer also bears a particular responsibility to ensure that all substances used in the preparation of radiopharmaceuticals are handled in a manner that ensures their freedom from pyrogens. Pyrogen tests are specified in certain monographs where there are special dangers.

Addition of bacteriostatic agents

Injections of radiopharmaceuticals are commonly supplied in containers that are sealed to permit the withdrawal of successive doses on different occasions. The *International Pharmacopoeia* normally requires that such injections should contain a suitable bacteriostatic agent in a suitable concentration.

Many common bacteriostatic agents (for example, benzyl alcohol) are gradually destroyed by the effect of radiation in aqueous solutions. The rate of destruction is dependent upon a number of factors, including the nature of the radionuclide and the radioactive concentration of the solution. It is, therefore, not always possible to prescribe an effective bacteriostatic agent for an injection of a radiopharmaceutical and for certain preparations the addition of an agent is undesirable; for this reason the inclusion of bacteriostatic agents is not mandatory. The nature of the bacteriostatic agent, if present, must be stated on the label; if no bacteriostatic agent is present, this must also be stated. Radiopharmaceuticals whose expiry periods are greater than one day and that do not contain a bacteriostatic agent should preferably be supplied in single-dose containers.

Other requirements

Radiopharmaceuticals administered parenterally should comply with the relevant requirements for injections in the *International Pharmacopoeia*, except that they are not subject to the requirements concerning volume of injection in a single-dose container.

Expiry date

The special nature of a radiopharmaceutical requires that it be assigned an expiry period (or an expiry date), beyond which its continued use is not recommended. The expiry period so designated begins with the date at which the radioactivity is expressed on the label, and may be stated in terms of days, weeks or months. For longer-lived radionuclides, the expiry period does not exceed 6 months. The expiry period depends on the radiochemical stability and the content of longer-lived radionuclidic impurity in the preparation under consideration. At the end of the expiry period, the radioactivity will have decreased

to the extent where insufficient radioactivity remains to serve the intended purpose or where the dose of active ingredient must be increased so much that undesirable physiological responses occur. In addition, chemical or radiation decomposition may have reduced the radiochemical purity to an unacceptable extent. Also the radionuclidic impurity content may be such that an unacceptable radiation dose would be delivered to the patient. The use of products beyond their expiry periods is, therefore, inadvisable.

Labelling

In general, the label should include:

(1) the name of the preparation;
(2) a statement that the product is radioactive;
(3) the name and location of the manufacturer;
(4) the total radioactivity present at a stated date and hour (whenever the half-life period is more than 30 days only the date need be stated);
(5) the expiry date or the expiry period;
(6) a number or other indication by which the history of the product may be traced (for example, batch or lot number);
(7) in the case of solutions, the total volume of the solution;
(8) special storage requirements with respect to temperature and light.

NOTE: In the case of a solution, instead of a statement of the total radioactivity, a statement of the radioactive concentration (for example, in mCi or MBq per ml of the solution) may be given.

The shipment of radioactive substances is subject to special national and international regulations as regards their packaging and outer labelling.

Storage

Radiopharmaceuticals should be kept in well-closed containers and stored in an area assigned for the purpose. The storage conditions should be such that the maximum radiation dose rate to which persons may be exposed is reduced to an acceptable level. Care should be taken to comply with national regulations for protection against ionizing radiation. Glass containers may darken under the effect of radiation.

Methods of Analysis

Methods of Analysis: Contents

Methods of Analysis

1. Physical and physicochemical methods

1.1 Measurement of mass

Tests that involve the measurement of mass require the use of balances of capacity and sensitivity corresponding to the degree of accuracy sought.

When weighing quantities of 50 mg or more that are to be "accurately weighed", an analytical balance of 100–200 g capacity and 0.1 mg sensitivity is required. When weighing quantities of less than 50 mg that are to be "accurately weighed", an analytical balance of 20 g capacity and 0.001 mg sensitivity, usually called an analytical microbalance, is required.

Balances of lower sensitivity are used for other tests in which a measurement of mass is involved.

Apparatus

Analytical balances should possess adequate capacity and sensitivity. They may be either of the equal-arm type, requiring the use of a set of calibrated weights, or of any other suitable type (for example, analytical microbalances using magnetic measurement) provided that their performance is periodically checked by means of a reference set of calibrated weights.

The analytical balance should be so constructed as to support its full capacity without developing undue stress and its sensitivity should not be altered by repeated weighings of the full-capacity load. It should preferably be equipped with a damping device (for example, a magnetic or air damper) that causes the beam to come quickly to rest (aperiodic balance).

The analytical balance may be constructed for manual placement of all weights or, preferably, be equipped with a weight-loading device for the whole or part of the balance range. In the latter case it should be equipped with loading registers clearly indicating the load applied. Furthermore, the analytical balance may be equipped with an optical scale projection system, usually encompassing a part of the balance range (e.g. where the displacement of the projected scale relative to the datum line gives a direct reading of weight), or a read-out device of any other type.

The type of analytical balance having constant sensitivity over the whole capacity range is the constant-load, single-pan balance. It has a set of weights suspended from a counterpoised beam; in the process of weighing, these are removed from the beam by a manually operated mechanical device until equilibrium is reached.

The analytical balance should be constructed in a proper housing with suitable openings to permit the placement of weighed material. The openings should be constructed in such a way as to exclude air currents. Desiccants may be placed inside the housing (e.g. silica gel, anhydrous calcium chloride) for the maintenance of a relatively dry atmosphere.

Sets of calibrated weights used with balances that require manual placement of weights and sets of weights used to check the sensitivity of balances of another type should be kept in a case made of suitable material and properly lined.

Placement of balance

The analytical balance should be placed upon a firm foundation that is as free from mechanical vibration as possible, preferably on an antivibration table of proper design. Alternatively, it may be placed on a concrete slab resting upon piers that are either sunk into the ground or connected to the construction elements of the building; or it may be placed upon a stout table or shelf protected by shock absorbers, such as cork mats or sheet rubber.

The balance should also be protected from humidity and acid fumes, preferably by placing it in a separate room of the laboratory. It should not be near a window or radiator, in direct sunlight, or in a position where draughts may come into contact with it.

The balance should be equipped with a levelling device and an indicator of proper position. Proper adjustment of levelling should be frequently checked.

Checking of sensitivity

The sensitivity of the balance should be periodically checked by a qualified expert.

Recommended procedure

Checking the stability of the equilibrium position

Before the balance is used, its equilibrium position without load should be checked several times. After each test, the balance has to be arrested.

The equilibrium position of the balance under load should also be determined from time to time, for example, with one-tenth of the full load and with the full load. The difference between equilibrium positions found in two successive determinations made with equal loads should not exceed 0.1 mg for analytical balances and 0.001 mg for analytical microbalances.

Operation of the balance

When the balance is not in use the balance beam and pan supports should be raised. The doors of the housing should always be kept closed.

To release the balance, the beam and pans should be lowered very carefully.

Objects to be weighed must be allowed to attain the temperature of the balance before weighing is started. The object to be weighed, as well as the weights, should always be placed on the pan as centrally as possible. During a weighing or on any occasion when objects are being added to or removed from the pans, both the beam arrests and the pan supports must be raised. Substances must be weighed in suitable containers such as beakers, weighing bottles, or crucibles. Liquids and volatile or hygroscopic solids must be weighed in tightly

closed vessels, such as stoppered weighing bottles. No chemicals or objects that might injure the balance pans should be placed directly upon them.

When small quantities of a substance (for example, the sulfated ash) must be weighed in a large vessel and a fairly long period elapses between the two weighings, atmospheric pressure and temperature may alter sufficiently to affect the buoyancy and thus cause an appreciable error. In two-pan balances, this error may be eliminated by using another vessel of similar shape and weight for taring.

The pans of the balance should be periodically lightly brushed with a camel-hair or similar brush to remove any dust that may have collected.

The weights should be handled only by means of a pair of forceps, which should possess tips covered with suitable material.

1.2 Determination of melting temperature, melting range, congealing point, boiling point, and boiling range

The thermodynamically true melting point of a substance (the triple point) is a physical constant that is indicative of the identity and purity of the material. It is defined as the temperature at which the solid, liquid, and gaseous phases of the substance are in equilibrium in an evacuated, closed system. Under normal atmospheric pressure the solid and liquid phases of a substance are in equilibrium at a temperature that differs somewhat from the triple point but, since pressure effects on the solid-liquid transition temperature are minimal, this difference does not, in general, exceed a few hundredths of a degree Celsius.

Methods for the determination of equilibrium melting points are laborious and require complicated equipment. The usual practice is, therefore, to estimate melting points by dynamic rather than equilibrium methods. The melting points thus determined usually differ significantly from the corresponding triple points. The magnitude of the deviation varies with the particular method employed, with the criterion adopted as the "melting point", and possibly with the substance under examination. Melting points determined by the capillary method of the *International Pharmacopoeia* are typically about one degree higher than the thermodynamically true melting points.

Determination of melting points (called subsequently melting temperatures) is used in pharmacopoeial specifications primarily for identification of the substance concerned. The validity of the identification is greatly enhanced if the so-called mixed-melting point procedure is applied. This involves an additional determination to demonstrate that the substance being examined and a mixture prepared of equal parts of this substance and an authentic specimen (reference substance) of the substance melt at the same temperature. If the two substances are not identical the mixture normally melts at a significantly lower temperature than the substance being examined, and the melting range is relatively broad.

The presence of impurities in a substance results in a more or less pronounced lowering of its melting point. Even more significant is that impurities present in the substance may cause its melting range to be extended. In most

cases where melting behaviour is used as a criterion of purity the *International Pharmacopoeia*, therefore, prescribes determination of the melting range rather than the melting point.

Similarly for liquids, determinations of boiling point and boiling range give information that contributes to the identification and purity estimation of liquid compounds. Practical considerations again dictate the use of methods that yield apparent constants that may differ from the thermodynamically true values. However, if the prescribed experimental conditions are closely adhered to, the results obtained are of sufficient reproducibility.

1.2.1 Melting temperature and melting range

A. Determination of melting temperature and melting range of pulverizable substances

The *melting range* of a substance is the range between the corrected temperature at which the substance begins to collapse or form droplets on the wall of a capillary tube and the corrected temperature at which it is completely melted as shown by the disappearance of the solid phase.

The statement in a monograph "melting range *a-b* °C" means that the melting range determined by the method below must fall within these limits.

The *melting temperature* of a substance is the corrected temperature at which it is completely melted as shown by the disappearance of the solid phase.

Apparatus

A suitable apparatus for the determination consists of a glass vessel with appropriate liquid, a controlled source of heat, a thermometer, a capillary tube, and a magnifying glass.

The glass vessel should have a suitable construction, contain an appropriate liquid and be fitted with a stirring device capable of rapid mixing of the liquid (certain liquid silicones are suitable). The controlled source of heat should be capable of raising the temperature of the liquid heating medium at the required rate.

Standardized thermometers should cover the range −10 to +360 °C, the length of one degree on the scale being not less than 0.8 mm. These thermometers should preferably be of the mercury-in-glass, solid-stem type with a cylindrical bulb and made of approved thermometric glass suitable for the range covered; each thermometer should have a safety chamber.

Thermometers used for determination of melting temperatures may be calibrated for total or partial immersion. A *total-immersion thermometer* should read correctly when it is immersed at least to the end of the liquid column in the medium, the temperature of which is to be measured. A *partial-immersion thermometer* should read correctly when it is immersed to a prescribed depth, and when the emergent liquid column is under prescribed conditions. When total-immersion thermometers are used partially immersed, an auxiliary thermometer is required for the determination of the emergent-stem correction. These

two thermometers should be surrounded with a glass tube above the surface of the heating material.

The capillary tube should be made of borosilicate glass, closed at one end, and have the following dimensions: thickness of the wall, about 0.10–0.15 mm; length, suitable for the apparatus used; internal diameter, 0.9–1.1 mm.

A suitable magnifying glass should be used for observation of the capillary tube.

Other apparatus or methods may be used provided they are capable of equal accuracy and have been calibrated against the method of the *International Pharmacopoeia* by means of the WHO Melting Point Reference Substances[1].

Recommended procedure

Spread a small quantity of the finely powdered substance in a thin layer and dry it in a vacuum desiccator over silica gel, desiccant, R, phosphorus pentoxide R, or other suitable desiccant for 24 hours, or at a temperature specified in the monograph.

Transfer a quantity of the dried powder to a dry capillary tube and pack the powder, for example, by tapping the tube on a hard surface, so as to form a tightly packed column about 3 mm in height. Introduce the capillary tube into the heated bath at a temperature 5 °C below the expected lower limit of the melting range, the rise of temperature being regulated beforehand to 1 °C per minute, unless either the temperature of the introduction of the capillary tube into the bath or the rate of temperature rise are otherwise specified in the monograph. The capillary tube should be fitted in the bath in such a way that its closed end is at the level of the middle of the bulb of the standard thermometer.

When a thermometer calibrated for partial immersion is used, care must be taken that it is immersed exactly to its immersion mark when the readings are taken.

Unless otherwise specified in the monograph, readings are taken of the temperature at which the substance is observed to collapse or form droplets on the wall of the tube and of the temperature at which it is completely melted as indicated by the disappearance of the solid phase.

To the temperature readings add the correction for deviation of the standard thermometer. When thermometers calibrated for total immersion are used partially immersed, add to the readings of the standard thermometer also the emergent-stem correction, which is obtained as follows:

> Before starting the determination of the melting range, an auxiliary thermometer is attached so that the bulb touches the standard thermometer at a point midway between the graduation for the expected melting point and the surface of the heating material. When the substance has melted the temperature is read on the auxiliary thermometer. The correction to be added

[1] A set of substances with melting points according to the *International Pharmacopoeia* in the temperature range +69 to +263 °C. The substances are available from the WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Produktion & Laboratorier, Centrallaboratoriet, ACL, Prismavägen 2, SE-141 75 Kungens Kurva, Sweden.

to the temperature reading of the standard thermometer is calculated from the following formula:

$$0.00015\ N(T - t)$$

where T is the temperature reading of the standard thermometer;
t is the temperature reading of the auxiliary thermometer;
N is the number of degrees of the scale of the standard thermometer between the surface of the heating material and the level of the mercury.

When needed, the emergent-stem correction for thermometers calibrated for partial immersion may be calculated from the same formula as above, but replacing T by T_s, which is the mean temperature of the emergent-stem of the thermometer at the time of calibration.

Both the above-mentioned corrections for emergent-stem and any deviation of the standard thermometer may conveniently be replaced by calibration of the apparatus by means of the WHO Melting Point Reference Substances.

B. Determination of melting point of fats, waxes, etc.

The melting point of fats, waxes, etc. is the corrected temperature at which the column of substance in the capillary tube becomes transparent or moves upwards, when tested by the method described below.

Apparatus

A similar apparatus to that described under A for the determination of melting temperature and melting range of pulverizable substances should be used with the following modifications:

— water should be used in the heating vessel;
— an accurately standardized thermometer should cover the range –10 to +100 °C;
— a glass capillary tube should have the same dimensions as described under A but be open at both ends; soft glass capillary tubes may be used.

Recommended procedure

Unless otherwise specified in the monograph, melt the substance at as low a temperature as possible, and then suck the liquid up to a height of about 10 mm in the capillary tube. Cool the charged tube at 10 °C or lower for 24 hours. If the monograph specifies that the melting temperature is to be determined without previous melting of the substance, charge the capillary tube by pushing it into the unmelted substance so that a column about 10 mm long is forced in. The determination may then be carried out immediately. Attach the tube to the thermometer in the water-bath by means of a rubber band or otherwise so that the lower end of the capillary tube is at the level of the middle of the bulb of the thermometer, and the distance between the lower end of the

capillary tube and the water level is about 20 mm. Heat the bath with constant stirring, the heating being regulated so that the temperature rise, at a temperature of 5 °C below the expected melting temperature, is about 1 °C per minute.

1.2.2 Congealing point

The congealing point of a liquid or of a melted solid is the highest temperature at which it solidifies. The congealing point of the liquid is the same as the melting temperature of the solid, but since the liquid may be cooled to a temperature below its congealing point without assuming the solid form, the method described below is used to determine the congealing point of a liquid or of a melted solid.

Apparatus
A suitable apparatus consists of a test-tube of about 2 cm internal diameter and about 10 cm in length, suspended by means of a bored cork inside a larger tube, about 3 cm in diameter and 12 cm in length, a vessel with water or suitable freezing mixture, and an accurately standardized thermometer.

Recommended procedure

Unless otherwise specified in the monograph, place in the inner test-tube about 10 ml of the liquid, or 10 g of the melted solid, to be tested and cool together the inner and the outer tubes in water or in a suitable freezing mixture to a temperature about 5 °C below the expected congealing point of the liquid; with the thermometer gently stir the liquid until it begins to solidify. At first there is a gradual fall in temperature. Then, as the solid phase forms, the temperature remains constant for some time or rises before becoming constant. The highest temperature observed is regarded as the congealing point. If the liquid does not start to congeal within 2 °C of the expected temperature, congelation may be induced by adding a small crystal of the substance to the liquid or by rubbing the inner walls of the test-tube with the thermometer.

1.2.3 Boiling point

The boiling point of a liquid is the corrected temperature at which the liquid boils under normal atmospheric pressure when determined by the method described below.

Apparatus
A suitable apparatus for the determination consists of a vessel with appropriate liquid, a source of heat, and a thermometer, as described under A for the determination of melting temperature and melting range for pulverizable substances; also needed are a thin-walled test-tube of glass of external diameter about 4 mm and length suitable for the apparatus used and a thin-walled cap-

illary tube of glass of internal diameter not exceeding 1 mm, which should be closed by fusing about 2 mm from one end.

Recommended procedure

Transfer 3–4 drops of the liquid to be tested (or the equivalent quantity of a solid compound) to the test-tube. Place the capillary tube (fused end down) in the test-tube and introduce the test-tube into the heating bath in such a way that its lower end is at the level of the middle of the bulb of the thermometer. Heat the bath rapidly with constant stirring to a temperature about 10 °C below the expected boiling point, then regulate the heating so that the temperature rise is 1–2 °C per minute. During the heating bubbles begin to escape from the lower end of the capillary tube, slowly at first but then more rapidly as the temperature approaches the boiling point. Read the temperature at which bubbles are released in an even rapid stream and then decrease the heating so that the temperature of the bath falls 1–2 °C per minute. Read the temperature at which the release of bubbles ceases. The boiling point is taken as the average of the two temperatures, corrections for emergent-stem of the thermometer and for deviation from normal atmospheric pressure being applied as necessary. Obtain the emergent-stem correction as described under A for the determination of melting temperature and melting range of pulverizable substances. If the determination is made at a barometric pressure that deviates from 101.3 kPa (760 mmHg), add to the temperatures the following correction:

$k(p - p_1)$

where p is the standard barometric pressure;
p_1 is the barometric pressure read on a mercury barometer, without taking into account the temperature of the air; and
k is the boiling temperature increment, as indicated below.

For pressures read on a barometer calibrated in kPa, use the following data:

$p = 101.3$
$k = 0.3$ (boiling temperature increment produced by a rise of pressure of 1 kPa), unless otherwise specified in the monograph.

For pressures read on a barometer calibrated in mmHg, use the following data:

$p = 760$
$k = 0.04$ (boiling temperature increment produced by a rise of pressure of 1 mmHg), unless otherwise specified in the monograph.

1.2.4 Boiling range

The boiling range (distillation range) is the corrected range of temperature, within which the whole or a specified portion of a liquid distils, under normal atmospheric pressure, when determined by the method described below.

Apparatus

A suitable apparatus for the determination consists of a distillation flask, a condenser, a receiver, a heat source with heat shields, and a thermometer.

The distillation flask of 50–60 ml capacity is made of heat-resistant glass. Flasks with the following dimensions are suitable: neck 10–12 cm long and 14–16 mm in internal diameter with a side-arm, 10–12 cm long and about 5 mm in internal diameter, attached at about the midway point of the neck and forming an angle of 70–75 ° with the lower portion of the neck.

The condenser is either a straight glass condenser, made of heat-resistant glass, 55–60 cm in length with a water jacket about 40 cm in length, or a condenser of another design having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or a bent adapter may be attached to it to serve as a delivery tube.

The receiver consists of a 25–50-ml measuring cylinder graduated in subdivisions of 0.5 ml.

The heat source consists of a small gas burner, preferably of a Bunsen type, or an electric heater or mantle capable of adjustment comparable to that possible with the gas burner. If a gas burner is used, a suitable shield is placed around the flask near its bottom. The shield is made of heat-resistant material in the form of a square with sides of 14–16 cm and with a perforation in the centre. The diameter of the latter should be such that when the flask is set into it, the portion of the flask below the upper surface of the shield has a capacity of 3–4 ml.

The thermometer should preferably be calibrated for partial immersion of 100 mm as described under A for the determination of melting temperature and melting range of pulverizable substances; otherwise a total-immersion thermometer may be used with appropriate emergent-stem correction. When the thermometer is placed in position, the stem should be located in the centre of the neck of the flask, and the top of the bulb should be just below the bottom of the outlet to the side-arm.

Recommended procedure

Place in the flask 25 ml of the liquid to be tested, taking care not to allow any of the liquid to enter the side-arm, and add 0.3–0.5 g of glass-beads or other suitable substance. Shield the burner and the flask from external air currents and apply heat so that the vapour rises only slowly into the neck of the flask and between 5–10 minutes elapse before the first drop of distillate falls from the condenser. Continue the distillation at the rate of 2–3 ml per minute, collecting the distillate in the receiver. Read the temperature when the first drop of distillate falls from the condenser, and again when the last quantity of liquid evaporates from the bottom of the flask or when the specified percentage has distilled over.

The boiling ranges (distillation ranges) indicated in the monographs are applied at a barometric pressure of 101.3 kPa (760 mmHg). If the determination is made at some other barometric pressure, correct the observed temperature readings for any difference in the barometric pressure by allowing 0.1 °C for

each 0.36 kPa (2.7 mmHg) of the difference, adding if the pressure is lower, or subtracting if the pressure is higher.

1.3 Determination of mass density and relative density

The mass density (ρ) of a substance is the mass of one unit volume of the substance. In terms of SI base units, mass density is expressed in kilograms per cubic metre. However, in the *International Pharmacopoeia* the mass density is expressed in kilograms per litre (which is equivalent to grams per millilitre) at a temperature of 20 °C (ρ_{20}) and it is corrected for buoyancy (i.e. reduced to vacuum conditions). For pharmacopoeial purposes the mass density of liquids is not measured directly but calculated from their relative density.

The relative density d_{20}^{20} is the ratio of the mass of the substance in air at 20 °C to that of an equal volume of water at the same temperature. The term "relative density" d_{20}^{20} is equivalent to the formerly used term "specific gravity determined at 20 °C".

The relative density d_4^{20} denotes the ratio of the mass of the substance in air at 20 °C to that of an equal volume of water at 4 °C. As the relative density of water at 20 °C is 0.998234, these values are related by the following equation:

$$d_4^{20} = 0.998234\ d_{20}^{20}$$

Recommended procedure

Determine the relative density (d_{20}^{20}) using a hydrostatic balance (only when the precision indicated in the monograph is three decimal digits) or a pycnometer.

If the value of mass density ρ_{20} (in kg/l or g/ml) is referred to in the monograph, carry out the measurement of relative density and from the value obtained calculate the mass density according to the formula:

$$\rho_{20} = 0.99703\ d_{20}^{20} + 0.0012$$

Use of a hydrostatic balance

Use an instrument of suitable construction placed on a horizontal support. The plummet (diver) should be suspended on a thin wire, made preferably of platinum. To calibrate the instrument equilibrate the plummet in the air, then immerse it in the cylinder filled with water and equilibrate again by placing suitable riders (weights) at appropriate notches along the beam. The plummet should swim freely in the liquid. Fill the cylinder with the test liquid and carry out the measurement in a similar way. Take care that the length of the immersed portion of the suspending wire is similar in all measurements. The weight that has to be added to obtain the equilibrium in the test liquid (or to be subtracted in the case of liquids of density lower than that of water) gives directly the measure of its relative density.

Use of a pycnometer

Use a pycnometer of suitable form of a capacity of not less than 5 ml. Weigh accurately the empty, dry pycnometer, and fill it with the test liquid brought previously to a temperature of about 20 °C. Hold the filled pycnometer at a temperature of 20 ± 1 °C for about 30 minutes, adjust the liquid to the mark using, if necessary, a small strip of filter-paper to remove the excess and to wipe the inlet from the inside, and weigh accurately. Calculate the weight of the liquid in the pycnometer. Remove the liquid, clean and dry the pycnometer, repeat the measurement with carbon-dioxide-free water R, also at 20 ± 1 °C, and calculate the weight of water in the pycnometer. The ratio of the weights of the test liquid and of water gives the relative density (d_{20}^{20}).

1.4 Determination of optical rotation and specific rotation

Many substances possess the inherent property to rotate the plane of incident polarized light; this property is called optical activity. The measurement of optical activity is used for pharmacopoeial purposes mainly to establish the identity of the substance. It may also be employed to test the purity of the substance (absence of optically non-active foreign substances) and as an assay procedure.

Optical rotation

The optical rotation is the angle through which the plane of polarization is rotated when polarized light passes through a layer of a liquid. Substances are described as dextrorotatory or levorotatory according to whether the plane of polarization is rotated clockwise or counterclockwise, respectively, as determined by viewing towards the light source. Dextrorotation is designated (+) and levorotation is designated (–).

In the *International Pharmacopoeia* the optical rotation (α) is expressed in angular degrees. In the SI, the angle of optical rotation is expressed in radians (rad).

The optical rotation is measured on a layer of suitable thickness at the wavelength specified in the monograph. If the sodium D line is specified, the sodium light of wavelength 589.3 nm (a mean value for a doublet at 589.0 nm and 589.6 nm) should be used. The wavelength of the mercury green line at 546.1 nm is also frequently used. If the wavelength specified lies in the ultraviolet range, the use of a photoelectric polarimeter is necessary.

The measurement of optical rotation should be carried out at the temperature indicated in the monograph, usually at 20–25 °C. Some substances have large temperature coefficients and for them special care should be taken to adjust the temperature indicated.

Specific optical rotation (specific rotation)

The specific optical rotation of a liquid substance is the angle of rotation measured as specified in the monograph, calculated with reference to a layer 100 mm thick, and divided by the relative density (specific gravity) measured at the temperature at which the rotation is measured.

The specific optical rotation of a solid is the angle of rotation measured as specified in the monograph, and calculated with reference to a layer 100 mm thick of a solution containing 1 g of the substance per ml.

$$\text{Specific rotation} = \frac{10000a}{lc} = \frac{10000a}{ldp}$$

where a is the observed rotation, l is the length of the observed layer in mm, c is the number of g of substance contained in 100 ml of solution, d is the relative density and p is the number of g of substance contained in 100 g of solution.

In the *International Pharmacopoeia* the specific optical rotation is expressed as $[\alpha]_{\lambda}^{t}$ where t is the temperature and λ the wavelength. For solid substances the solvent, if different from water, and the concentration are further described. The general directions concerning wavelength and temperature as given above for optical rotation refer equally to the measurement of specific optical rotation. In the SI specific optical rotation (optical rotatory power) is given in $m^2 \cdot$ rad/kg and molar optical rotatory power (α_m) in $m^2 \cdot$ rad/mol.

Apparatus

Optical rotation is measured with a polarimeter. The zero point of the polarimeter is determined with the tube empty but closed for liquid substances and filled with the specified solvent for solutions of solid substances.

Generally, a polarimeter accurate to 0.05° of angular rotation, and capable of being read with the same precision, suffices for pharmacopoeial purposes; in some cases, a polarimeter accurate to 0.01° of angular rotation, and read with comparable precision, may be required.

Polarimeters for visual measurement: commercial instruments are normally constructed for use with sodium light or a mercury-vapour lamp. The manufacturer's instructions relating to a suitable light source should be followed.

Photoelectric polarimeters: where it is directed in the individual monograph to determine the optical rotation photoelectrically, use a photoelectric polarimeter capable of an accuracy of at least 0.01°.

Measurement of optical rotation

The accuracy and precision of optical rotatory measurements will be increased if they are carried out with due regard for the following general considerations.

Optical elements of the instrument must be brilliantly clean and in exact alignment. The match point should lie close to the normal zero mark. The light source should be rigidly set and well aligned with respect to the optical bench. It should be supplemented by a filtering system capable of transmitting light of a sufficiently monochromatic nature. Precision polarimeters are generally designed to accommodate interchangeable discs to isolate the D line from sodium light or the 546.1 nm line from the mercury spectrum. With polarimeters not thus designed, cells containing suitably coloured liquids may be employed as filters.

Observations should be accurate and reproducible to the extent that differences between replicates, or between observed and true values of rotation (the latter value having been established by calibration of the polarimeter scale with suitable standards), shall not exceed one-fourth of the range given in the individual monograph for the rotation of the substance being tested.

Polarimeter tubes should be filled in such a way as to avoid creating or leaving air bubbles, which interfere with the passage of the beam of light. Interference from bubbles is minimized by the use of tubes in which the bore is expanded at one end. However, with tubes of uniform bore, such as semi-micro or micro tubes, care is required for proper filling.

In closing tubes having removable end-plates fitted with gaskets and caps, the latter should be tightened only enough to ensure a leak-proof seal between the end-plate and the body of the tube. Excessive pressure on the end-plate may set up strains that result in interference with the measurement. In determining the optical rotation of a substance of low rotatory power, it is desirable to loosen the caps and tighten them again between successive readings in the measurement of both the rotation and the zero point. In this way, differences arising from end-plate strain will generally be revealed and appropriate adjustments to eliminate the cause can be made.

The requirements for optical rotation and specific rotation apply to the dried, anhydrous, or solvent-free material in all those monographs in which standards for loss on drying, water, or solvent content are given. In calculating the result, the loss on drying, water, or solvent content determined by the method specified in the monograph should be taken into account.

Recommended procedure

If the substance is a solid, weigh a suitable portion and transfer it to a volumetric flask by means of water, or other solvent if specified in the monograph, reserving a portion of the solvent for the blank determination. Add enough solvent to bring the meniscus close to, but still below, the mark, and adjust the temperature of the flask contents by suspending the flask in a constant-temperature bath. Add solvent to the mark, and mix. Transfer the solution to the polarimeter tube, preferably within 30 minutes from the time the substance was dissolved, taking care to standardize the elapsed time in the case of substances known to undergo racemization or mutarotation. During the elapsed time interval, maintain the solution at the required temperature.

If the substance is a liquid, adjust its temperature, if necessary, and transfer it directly to the polarimeter tube.

When a polarimeter is used for visual measurement, make at least 6 readings of the observed rotation at the required temperature. Take half the readings in a clockwise and the other half in a counterclockwise direction. Substitute the reserved solvent for the solution, and make an equal number of readings on it. In the case of liquid substances, carry out blank determinations on the empty, dry tube. The zero correction is the average of the blank readings, and is sub-

tracted from the average observed rotation if the two figures are of the same sign, or added if they are opposite in sign, to give the corrected observed rotation.

When a photoelectric polarimeter is used, a smaller number of readings is required, depending on the type of instrument.

1.5 Determination of refractive index

The refractive index (n) of a substance is the ratio of the velocity of light in a vacuum to its velocity in the substance. It varies with the wavelength of the light used in its measurement and with the temperature. It is, therefore, necessary to specify these conditions (n_λ^t). In practice it is usually convenient to measure the refraction with respect to air and the substance, rather than with respect to a vacuum and the substance, since, for pharmacopoeial purposes, this has no significant influence on the observed values.

The refractive index may also be defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction.

The measurement of the refractive index is employed for pharmacopoeial purposes mainly to establish the identity of liquid substances. It may also be used to test the purity of such substances.

Refractive indices are usually stated in terms of sodium light of wavelength 589.3 nm (line D) at a temperature of 20 ± 0.5 °C (n_D^{20}).

The accuracy of the measurement should be related to the requirements of the monograph. For pharmacopoeial purposes it is usually adequate to express the refractive index to three decimal places.

Apparatus

Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the sodium light of wavelength 589.3 nm (line D).

The optical parts of the apparatus should be kept brilliantly clean. The working surfaces of prisms should be free from scratches.

Subject to the directions given above, the manufacturer's instructions relating to a suitable light source should be followed.

The instrument should be calibrated against a standard provided by the manufacturer; the temperature control of the liquid being examined and the cleanliness of the prism should be checked frequently by determining the refractive index of distilled water, which is 1.3330 at 20 °C and 1.3325 at 25 °C.

1.6 Spectrophotometry in the visible and ultraviolet regions

Absorption spectrophotometry is the measurement of the absorption, by substances, of electromagnetic radiation of definite and narrow wavelength range, essentially monochromatic.

The spectral range used in the measurements described below extends from the short wavelengths of the ultraviolet through the visible region of the spectrum. For convenience of reference, this range may be regarded as consisting of two regions, the ultraviolet (190–380 nm) and the visible (380–780 nm).

Spectrophotometry in the visible region (formerly the term colorimetry was commonly used) is the measurement of absorption of visible light, which is usually not monochromatic but restricted by the use of pigmented or interference filters.

The ultraviolet and visible spectra of a substance generally do not have a high degree of specificity. Nevertheless, they are highly suitable for quantitative assay, and for many substances they are useful as additional means of identification.

General agreement has not yet been reached on the definition of terms used in spectrophotometry. The terms in italics used in connexion with spectrophotometric tests in the *International Pharmacopoeia* are defined as follows:

Absorbance (A) – The logarithm, to the base 10, of the reciprocal of the transmittance (T). The term internal transmission density may be used as a synonym of absorbance; descriptive terms formerly used included optical density, absorbancy, and extinction.

Transmittance (T) – The ratio of the radiant flux transmitted by the test substance to that of the incident radiant flux. Terms formerly used include transmittancy and transmission.

Absorptivity (a) – The quotient of the absorbance (A) divided by the product of the concentration of the substance (c), expressed in g per litre, and the absorption path length (b) expressed in cm ($a = A/bc$). Two terms closely related to absorptivity are specific extinction and specific absorption coefficient. The term "specific extinction" ($E^{1\%}_{1\,cm}$), as generally used in pharmacopoeias, denotes the quotient of the absorbance (A) divided by the product of the concentration of the substance (c), expressed in g per 100 ml, and the absorption path length (b) expressed in cm; therefore $E^{1\%}_{1\,cm} = 10a$. The term "specific absorption coefficient", tentatively proposed by the Commission on Physicochemical Symbols, Terminology and Units of the International Union of Pure and Applied Chemistry (IUPAC), is defined as the quotient of absorbance (A) divided by the product of concentration (c) and the absorption path length (l); when the symbol a_{SI} is used for specific absorption coefficient, which in the SI should be expressed in m^2 per kg, $a_{SI} = 100a$. The term "absorptivity" is not to be confused with absorbancy index or extinction coefficient.

Molar absorptivity (ε) – The quotient of the absorbance (A) divided by the product of the concentration of the substance (c), expressed in moles per litre, and the absorption path length (b) in cm. It is also the product of the absorptivity (a) and the molecular weight of the substance. The term "molar absorption coefficient (linear)" recommended by the Commission on Physicochemical Symbols, Terminology and Units of IUPAC is defined as the quotient of the internal transmission density (absorbance)

of the substance divided by the product of the concentration of the substance and the absorption path length, and according to the SI should be expressed in m^2 per mole. The terms formerly used for molar absorptivity include molar absorbancy index and molar extinction coefficient.

Absorption spectrum – The relationship of absorbance and wavelength or any functions of these, frequently represented in a graphic form.

The use of absorption spectrophotometry in the visible and ultraviolet regions for assay procedures is based on the fact that the absorptivity of a substance is usually a constant independent of the intensity of the incident radiation, the internal cell length, and the concentration, with the result that concentration may be determined photometrically.

Deviations from the above may be caused by either physical, chemical, or instrumental variables. Deviations due to instrumental error might be caused by slit-width effects, stray light, or by polychromatic radiation. Apparent failure may also result from a concentration change in solute molecules because of association between solute molecules or between solute and solvent molecules, or dissociation or ionization.

Apparatus

In essence all types of spectrophotometer are designed to permit substantially monochromatic radiant energy to be passed through the test substance in a suitable form and to allow measurement of the fraction of that energy that is transmitted. The spectrophotometer comprises an energy source, a dispersing device with slits for selecting the wavelength band, a cell or holder for the test substance, a detector of radiant energy, associated amplifiers, and measuring and recording devices. Some instruments are manually operated, while others are equipped for automatic operation. Instruments are available for use in the visible region of the spectrum, usually 380 nm to about 700 nm, and in the visible and ultraviolet regions of the spectrum, usually 190 nm to about 700 nm.

Both double-beam and single-beam instruments are commercially available and either is suitable. Depending on the type of apparatus used, the results may be displayed on a scale, on a digital counter, or by a recorder or printer.

The apparatus should be maintained in proper working condition. The housing of the optical system should minimize any possibility of errors due to stray light; this is particularly relevant in the short-wave region of the spectrum.

Cells usually used in the spectral range discussed are 1-cm absorption cells with glass or silica windows. Other path lengths may also be used. The cells used for the test solution and the blank should be matched, and must have the same spectral transmittance when containing only the solvent. If this is not the case, an appropriate correction must be applied.

Spectrophotometer calibration

Spectrophotometers should be regularly checked for accuracy of calibrations. Where a continuous source of radiant energy is used, both the wavelength and photometric scales should be calibrated; where a spectral line source is used, only the photometric scale need be checked.

A number of sources of radiant energy have spectral lines of suitable intensity, adequately spaced throughout the spectral range selected. The exact values for the position of characteristic lines in quartz-mercury arc are 253.7, 302.25, 313.16, 334.15, 365.48, 404.66 and 435.83 nm. The wavelength scale may also be calibrated by means of suitable glass filters that have useful absorption bands through the visible and ultraviolet regions. Standard glass containing didymium (a mixture of praseodymium and neodymium) has been widely used. Glass containing holmium is considered superior. The exact values for the position of characteristic maxima in holmium glass filters are 241.5 ± 1, 287.5 ± 1, 360.9 ± 1 and 536.2 ± 3 nm. Holmium glass filters are obtainable from some national institutions and from commercial sources. The performance of an uncertified filter should be checked against one that has been properly certified. The wavelength scale may also be calibrated using holmium perchlorate TS. The exact values for the position of characteristic maxima of this solution are as follows: 241.15 nm, 278.2 nm, 361.5 nm and 536.3 nm. It should be noted that the position of characteristic maxima of holmium perchlorate solutions and holmium glass filters may differ slightly.

For the calibration of the photometric scale the tolerance generally permitted is ±1% of the absorptivity. For checking this scale potassium dichromate TS may be used. The exact values of absorbance and specific extinction for a solution of potassium dichromate containing exactly 60.06 mg in 1000 ml of sulfuric acid (0.005 mol/l) VS at an absorption path length of 1.000 cm and the permitted tolerances for *A* are given below:

Wavelength	**235 nm (minimum)**	**257 nm (maximum)**	**313 nm (minimum)**	**350 nm (maximum)**
A	0.748	0.865	0.292	0.640
Permitted tolerance	0.740–0.756	0.856–0.874	0.289–0.295	0.634–0.646
$E^{1\%}_{1\,cm}$	124.54	144.02	48.62	106.56

A number of standard inorganic glass filters of known transmittance produced for checking the photometric scale are also available from some national institutions and from commercial sources but may require periodic calibration.

Operation of spectrophotometers

Detailed instructions for operating spectrophotometers are supplied by the manufacturers. To achieve significant and valid results, the operator of a spectrophotometer should be aware of its limitations and of potential sources of error and variation. The instruction manual should be followed closely on such matters as care, cleaning, and calibration of the instrument, and instructions for

operation. Where double-beam recording instruments are used the cell containing solvent only is placed in the reference beam.

The cleanliness of absorption cells should receive particular attention. Usually, after treatment with an appropriate cleansing medium, the cells should be rinsed with distilled water and then with a volatile organic solvent to promote drying. Test solutions should not be left in the cells longer than necessary for carrying out the measurement. When handling the cells, special care should be taken never to touch the external surfaces through which the light beam passes. When the solvent and the test solution are transferred to the cells, care is to be taken that the liquids do not contaminate the outer surfaces.

Solvents for use in the ultraviolet region

Many solvents are suitable for tests and assays using spectrophotometry in the ultraviolet region. Water, alcohols, chloroform, lower hydrocarbons, ethers, and dilute solutions of ammonium hydroxide, sodium hydroxide, sulfuric acid and hydrochloric acid can be used for this purpose. The solvents differ as to the lower wavelength at which the decrease in transparency prevents their use. Precautions should be taken to use solvents free from contaminants absorbing in the relevant spectral region. Specially purified solvents for spectrophotometric determinations are available commercially from several sources but need only be used when the spectral characteristics of the usual analytical grade of solvent are inadequate for a particular purpose.

The absorbance of the solvent cell and its contents should not exceed 0.4 per cm of path length when measured with reference to air at the same wavelength. The solvent in the solvent cell should be of the same batch as that used to prepare the solution and must be free from fluorescence at the wavelength of measurement. Ethanol (~750 g/l), dehydrated ethanol, methanol, and cyclohexane used as solvents should have an absorbance, measured in a 1-cm cell at 240 nm with reference to water, not exceeding 0.10.

Identification tests in the ultraviolet region

The monographs describing qualitative tests involving spectrophotometry in the ultraviolet region specify the concentration of the solution and the path length. In such tests it is more convenient to use a recording instrument. If the conditions stated are not appropriate for a particular instrument, the thickness of the solution should be varied and not the concentration.

Some identification tests involving spectrophotometry require the use of reference substances, generally an International Chemical Reference Substance (ICRS). The reference substance is then to be prepared and simultaneously measured under conditions identical for all practical purposes to those used for the test substance. Unless otherwise specified in the monograph, in making up the solution of the reference substance, a solution of about the desired concentration (i.e. within 10% of the value) should be prepared. Identical conditions for the measurement include the following: wavelength setting, slit-width adjustment, cell placement, and correction and transmittance levels.

A useful approach for identification tests in the ultraviolet region is to quote the ratio of absorbance values at two maxima. This procedure minimizes the influence of instrumental variations on the test and obviates the need for a reference substance.

Quantitative determinations in the ultraviolet region

Spectrophotometric assays usually call for a comparison of the absorbance produced by the solution of the test substance, prepared as specified in the monograph, with the absorbance of a solution of a reference substance. In such cases the spectrophotometric measurements are made first with the solution prepared from the reference substance and secondly with the solution prepared from the substance to be examined. The second measurement is carried out as quickly as possible after the first, using the same experimental conditions.

Spectrophotometric assays are usually carried out at a peak of spectral absorption for the compound concerned. The monographs give the commonly accepted wavelength for peak spectral absorption of the substance in question. It is known that different spectrophotometers may show minor variations in the apparent wavelength of this peak. Good practice demands that use be made of the peak wavelength actually found in the individual instrument, rather than the specific wavelength given in the monograph, provided the two do not differ by more than ±0.5 nm in the 240–280 nm range, by more than ±1 nm in the 280–320 nm range, and by more than ± 2 nm above 320 nm. If the difference is greater, recalibration of the instrument may be indicated.

The solution of the reference substance, generally an ICRS, is to be prepared and measured in the same manner as described for "Identification tests in the ultraviolet region". The calculations should be made on the basis of the exact amount weighed and, if the reference substance used has not previously been dried, on the dried or anhydrous basis. Specific instructions in individual monographs indicate the manner in which a reference substance is to be dried or treated prior to use. These instructions are to be followed unless otherwise specified in the individual test or assay, or in the labelling.

To ensure that the conditions used are appropriate the monograph may also specify the value of absorbance of a 1-cm layer of the reference substance. In this case the determination carried out against the reference substance is considered valid when the observed value of absorbance is within the range of values specified in the monograph.

For quantitative work, a manually scanning instrument is frequently used. When a recording instrument is used for that purpose special attention should be paid to proper calibration of the absorbance scale at the wavelength used.

Quantitative determinations are usually carried out at wavelengths above 235 nm. If the measurements are to be made at a wavelength in the 190–210 nm range, special precautions should be observed, for example, purging the cell compartment with nitrogen, use of solvents of special spectrophotometric quality, and using cells that are transparent in this region.

When measuring the absorbance at an absorption maximum the spectral slit-width must be small compared with the half width of the absorption band, otherwise an erroneously low absorbance will be measured. Particular care is needed for certain substances and the instrumental slit-width used should always be such that further reduction does not result in an increased absorbance reading. Problems may be encountered, owing to diffraction of the light beam, at slit-widths below 0.01 mm.

When the assays are carried out with routine frequency it is permissible to omit the use of a reference substance and use instead a suitable standard curve prepared with the respective ICRS. This may be done when, for the substance tested, the absorbance is proportional to the concentration within the range of about 75–125% of the final concentration used in the assay. Under these circumstances, the absorbance found in the assay may be interpolated on the standard curve, and the assay result calculated therefrom. Such standard curves should be confirmed frequently, and always when a new apparatus or new lots of reagents are put into use. In the event of uncertainty or dispute, direct comparison with an ICRS must be made.

Quantitative determinations in the visible region

Spectrophotometric assays in the visible region, as in the ultraviolet region, usually call for simultaneous comparison of the absorbance produced by the assay preparation with that produced by a standard preparation containing approximately an equal quantity of a reference substance.

For spectrophotometric assays in the visible region the recommendations given under "Quantitative determinations in the ultraviolet region", including those concerning the use of standard curves, should be followed with suitable modifications, where necessary. In this region, observed wavelengths should not differ by more than 5 nm from that specified in the monograph.

1.7 Spectrophotometry in the infrared region

The infrared region of the electromagnetic spectrum used in pharmaceutical analysis covers the range 4000–250 cm^{-1} (2.5–40 μm).[1]

Spectrophotometric measurements in the infrared region are used mainly as an identification test. The infrared spectrum is unique for any given chemical compound with the exception of optical isomers, which have identical spectra in solution. Polymorphism and other factors, such as variations in crystal size and orientation, the grinding procedure, and the possible formation of hydrates may, however, be responsible for a difference in the infrared spectrum of a given compound in the solid state. The infrared spectrum is usually not greatly affected by the presence of small quantities of impurities (up to several percent) in the tested substance. For identification purposes the spectrum may be com-

[1] The values given in brackets are wavelengths; the preceding values are wave-numbers, which are the reciprocals of the wavelengths.

pared with that of a reference substance, concomitantly prepared or with a standard reference spectrum.

The terms *absorption spectrum, absorbance, transmittance, absorptivity,* and *specific extinction* are described in 1.6 Spectrophotometry in the visible and ultraviolet regions.

Apparatus

Spectrophotometers for the infrared region are basically similar to those used for the visible and ultraviolet regions of the spectrum, but may differ as to the energy sources, optical materials, and detection devices. Furthermore, in some instruments the monochromator may be located between the test substance and the detector.

Spectrophotometers suitable for use for identification tests should operate in the range 4000–670 cm^{-1} (2.5–15 μm). They should be checked frequently to ensure that they meet the standards of performance laid down by the manufacturer of the instrument, including the reliability of the wavelength scales, which should be checked by use of a polystyrene film.

For the use of the attenuated total reflectance technique, the instrument should be equipped with a suitable attachment, which may be a single-reflection or a multi-reflection one. The attachment consists of a reflecting element and a suitable mounting permitting its alignment in the spectrophotometer for maximum transmission.

Use of solvents

The solvent used in infrared spectrophotometry must not affect the material, usually sodium chloride, of which the cell is made.

No solvent in appreciable thickness is completely transparent throughout the infrared spectrum. Carbon tetrachloride R is practically transparent (up to 1 mm in thickness) from 4000 to 1700 cm^{-1} (2.5 to 6 μm). Chloroform R, dichloromethane R, and dibromomethane R are other useful solvents. Carbon disulfide IR (up to 1 mm in thickness) is suitable as a solvent to 250 cm^{-1} (40 μm), except in the 2400–2000 cm^{-1} (4.2–5.0 μm) and the 1800–1300 cm^{-1} (5.5–7.5 μm) regions, where it has strong absorption. Its weak absorption in the 875–845 cm^{-1} (11.4–11.8 μm) region should also be noted. Other solvents have relatively narrow regions of transparency.

Preparation of the substance to be tested

To determine the infrared absorption spectrum of a substance, the latter has to be suitably prepared. Liquid substances may be tested directly or in a suitable solution. For solid substances, the usual methods of preparation include dispersing the finely ground solid specimen in mineral oil, or incorporating it in a transparent disc or pellet obtained by mixing it intimately with previously dried potassium halide and pressing the mixture in a die, or preparing a solution in a suitable solvent. Preparation of the substance for the attenuated total reflectance technique is described separately.

The following procedures may be used for the preparation of the substance:

Method 1. Use a capillary film of the liquid held between two sodium chloride plates or a filled cell of suitable thickness.

Method 2. Triturate a small quantity of the substance with the minimum amount of a suitable mineral oil or other suitable liquid to give a smooth, creamy paste; 2–5 mg of the substance being tested is often sufficient to prepare a satisfactory mull, which should be semi-transparent to light. Compress a portion of the mull between two flat sodium chloride or other suitable plates.

Method 3. Triturate the solid substance with dry, finely powdered potassium halide (potassium bromide IR, potassium chloride IR); the proportion of substance to the halide should be about 1 to 200 –, for example, 1.5 mg in 300 mg of the halide – in the case of prism instruments, and about 1 to 300 – for example, 1.0 mg in 300 mg of the halide – in the case of grating instruments. The amount taken should be such that the weight of substance per area of the disc is about 5–15 µg per mm^2, varying with the molecular weight and to some degree with the type of apparatus used. Insert a portion of the mixture in a special die and subject it under vacuum to a high pressure. Commercial dies are available and the manufacturer's instructions should be followed. Mount the resultant disc in a suitable holder. Several factors, for example, inadequate or excessive grinding, moisture or other impurities in the halide carrier, may give rise to unsatisfactory discs. Unless its preparation presents particular difficulties, a disc should be rejected if visual inspection shows lack of uniformity or if the transmission at about 2000 cm^{-1} (5 µm) in the absence of a specific absorption band is less than 75% without compensation.

Method 4. Prepare a solution of the liquid or solid substance in a suitable solvent and choose a concentration and cell thickness to give a satisfactory spectrum over a sufficiently wide wavelength range.

Identification by reference substance

Prepare the substance under examination and the reference substance by the same method, and record the spectrum of each from about 4000 to 670 cm^{-1} (2.5 µm to 15 µm) on an infrared spectrophotometer. The concentration of the substance should be such that the strongest peak attributable to the substance reaches to between 5% and 25% transmittance.

If the positions and relative intensities of the absorbance maxima in the spectrum of the substance under examination are not concordant with those of the spectrum of the reference substance in the case of the curves obtained by methods 2 or 3, this may be due to differences in crystalline form. When such difficulties are suspected, the substance should, where possible, be examined in solution. If examination in solution is not practicable, attempts should be made to obtain, by recrystallization, the reference substance and the substance under examination in the same crystalline form.

If the spectrum of mineral oil used in Method 2 interferes with regions of interest, an additional dispersion of the substance in a medium such as a suitable fluorinated hydrocarbon oil or hexachlorobutadiene may be prepared and the spectrum recorded in the regions where the mineral oil shows strong absorption.

Identification by reference spectrum

Prepare the substance being examined exactly as described in the note accompanying the International Reference Spectrum and record the spectrum from about 4000 to 670 cm^{-1} (2.5 to 15 μm) using an instrument that is being checked frequently to ensure that it meets the standards of performance laid down by the manufacturer. To allow for a possible difference in wavelength calibration between the instrument on which the International Reference Spectrum was obtained and that on which the spectrum of the substance is to be recorded, reference absorbance maxima of a polystyrene spectrum are superimposed on the International Reference Spectrum at about 2851 cm^{-1} (3.51 μm), 1601 cm^{-1} (6.25 μm) and 1028 cm^{-1} (9.73 μm). Similar reference maxima should be superimposed on the spectrum of the substance. Taking these polystyrene maxima into account, the identification is considered to be positive if the principal absorbance maxima in the spectrum of the substance being tested are concordant with the corresponding maxima in the relevant International Reference Spectrum. When comparing the two spectra, care should be taken to allow for the possibility of differences in resolving power between the instrument on which the International Reference Spectrum was prepared and the instrument being used to examine the substance. An International Reference Spectrum of polystyrene that was recorded on the same instrument as the collection of International Reference Spectra should be used for assessing these differences. It should be noted that the greatest variation due to differences in resolving power is likely to occur in the region between 4000 and 2000 cm^{-1} (2.5 and 5 μm).

Attenuated total reflectance technique

To determine the infrared absorption spectrum of a substance by the attenuated total reflectance technique the solid substance has usually to be finely pulverized. The powder may then be either packed directly against the prism of the attachment or adhesive tape may be used to facilitate the contact. The powdered substance is spread on the adhesive side of an adhesive tape to form an almost translucent layer and the tape is pressed on the reflecting element, on the side with powder in contact. Next, either the backing plate is attached or moderate pressure is applied in a suitable clamp for 1–2 minutes. Finally, the reflecting element is placed in the holder. The tape used in the procedure should preferably contain a natural rubber adhesive. In the case of some plastic materials it may be placed directly on the reflecting element.

Proper alignment of the attachment in the apparatus should be carefully controlled.

1.8 Atomic spectrometry: emission and absorption

Atomic emission spectrometry, flame photometry, and atomic absorption spectrometry are analytical techniques that measure the concentration of chemical elements in a sample. When elements are transformed into atomic vapour at high temperatures, emission or absorption of light may occur and this can be accurately measured at a unique resonant wavelength, which is characteristic of the emission/absorption lines of the elements concerned.

Using this basic process, the concentrations of most elements may be estimated by measuring the amount of radiation either emitted by the sample (emission spectrometry) or absorbed by the sample following production by a primary radiation source (absorption spectrometry).

In emission spectrometry/flame photometry, a small proportion of the atoms undergo excitation with a subsequent and characteristic emission of radiation, at wavelengths in the ultraviolet or visible regions, which is proportional to the number of excited atoms.

Thus under controlled conditions, it is possible with either technique to relate the measured light intensity (emission or absorption) to the concentration of individual elements in the sample.

The major advantage of emission spectrometry is the relatively low cost and simplicity of instrumentation, since it requires no additional source of primary radiation. The fraction of excited atoms produced by most elements at normal flame temperatures is small and hence, in practice, emission spectrometry is limited to a few elements such as sodium, potassium, lithium, and calcium. Sensitivity may be increased, however, by using higher flame temperatures or other more effective means of excitation.

In comparison, absorption spectrometry offers greater sensitivity, since absorption is proportional to the concentration of ground state atoms, which are produced in sufficient numbers by most elements at normal operating flame temperatures. The disadvantage of this technique is the high initial cost of instrumentation, because of the requirement for a primary source of radiation (an individual hollow cathode lamp, one for each element to be determined).

Both techniques are comparative in nature and require the concomitant preparation and use of standard reference solutions of all elements to be determined.

Apparatus

The emission spectrometer consists essentially of a burner – an atomic generator of the element to be determined (flame, furnace, plasma, arc, etc.), a suitable filter or monochromator, and a detector. The absorption spectrometer also includes a source of radiation (a hollow cathode tube) where the cathode consists of the same element as that to be determined.

Use of solvents

For flame spectrometry, the ideal solvent is one that produces neutral atoms and interferes the least with the emission or absorption processes. Differences

in surface tension or viscosity between the test and reference solutions may cause difficulties in the aspiration or atomization rates and significant changes in the signals generated. The solvent of choice for the preparation of test and reference solutions should, therefore, be water, although organic solvents, such as flammable solvents, either alone or mixed with water, may also be used if precautions are taken to ensure that they do not interfere with the stability of the flame. When mineral acids are necessary for the dissolution of the element, care should be taken to avoid interference from the acidic anion. A dilute solution of hydrochloric acid is the preferred solvent for this purpose.

Calibration

To calibrate the instrument, introduce water or the blank solution into the atomic vapour generator (flame) and adjust the reading of the instrument, either to zero for *emission spectrometers* or to indicate maximum transmission for *absorption spectrometers*. For emission measurements, introduce the most concentrated standard solution into the flame and adjust the sensitivity to full-scale deflection. Refer to the manufacturer's instructions for instruments with an absorbance scale.

Recommended procedure

Follow the manufacturer's instructions for the operation of the spectrometer, and use the wavelength indicated in the individual monograph. Use Method 1, unless otherwise specified in the monograph.

Method 1: External standard method

Prepare the solution of the substance to be tested as specified in the monograph. Prepare concurrently, adding any reagents in the same concentration as for the solution above, not fewer than three reference solutions of the element to be determined that cover the expected concentration range of the solution under test. Similarly, prepare a blank solution.

After calibration, introduce each reference solution into the instrument three times, recording the steady reading obtained. If the generator is a flame, wash the apparatus after each introduction with water or the blank solution to check that the reading returns to its initial setting. If the generator is a furnace, fire it after each introduction. Prepare a curve by plotting the mean of each group of three readings against the concentration.

Introduce the solution to be tested into the instrument three times, and record the readings. Determine the concentration of the element using the mean of the readings and interpolating from the curve.

Method 2: Standard addition method

Prepare in at least three similar volumetric flasks a series of solutions containing equal quantities of the substance to be tested as specified in the monograph and increasing volumes of the reference solution containing known concentra-

tions of the element to be determined. The concentrations chosen should be expected to give responses in the linear part of the curve. One of the solutions of the substance to be tested should contain no added reference solution.

After calibration, introduce each solution into the instrument three times, recording the steady reading obtained. If the generator is a flame, wash the apparatus after each introduction with water or the blank solution to check that the reading returns to its initial setting. If the generator is a furnace, fire it after each introduction.

By using a least-squares fit, calculate the linear equation of the graph and derive from it the concentration of the element determined in the test solution. Alternatively, plot the mean of the readings on a graph against the added quantity of the element and determine the concentration of the element in the test solution by extrapolating the straight line joining the points on the graph to an extended concentration axis.

1.9 Fluorescence spectrophotometry

Fluorescence spectrophotometry is the measurement of the fluorescence, i.e. photoluminescence, emitted by a substance while it is being exposed to ultraviolet, visible, or other electromagnetic radiation. In general, the light emitted by fluorescent solutions is of maximum intensity at a wavelength longer than that of the absorption band causing excitation, usually by some 20 or 30 nm.

The intensity of the light emitted by a fluorescent solution is, in certain circumstances, a simple function of the concentration of the solute and can, therefore, be used for analysis. It is difficult, however, to measure absolute fluorescence intensity, and measurements are usually made by reference to dilutions of a properly selected reference substance. The general scheme in fluorescence spectroscopy is, therefore, to excite with radiation at the wavelength of maximum absorption, and to measure or compare the intensity of the fluorescent light with that of a reference solution. The fluorescent light should be carefully freed from scattered incident light.

Terms

Fluorescence intensity is an empirical expression of fluorescence activity, commonly given in terms of arbitrary units proportional to detector response.

The *fluorescence emission spectrum* is the relationship between the intensity of the emitted radiation and the wavelength and is frequently represented in a graphic form.

The *fluorescence excitation spectrum* is the relationship between the maximum intensity of radiation emitted by an activated substance and the wavelength of the incident radiation and is frequently represented in a graphic form.

Apparatus

Measurement of fluorescence intensity can be made with a simple filter fluorimeter (sometimes the term fluorometer is used). Such an instrument consists

of a radiation source, a primary filter, a sample chamber, a secondary filter, and a fluorescence detection system. In most such fluorimeters, the detector is placed on an axis at 90° from that of the incident beam. This right-angle geometry permits the incident radiation to pass through the test solution without contaminating the output signal received by the fluorescence detector. However, the detector unavoidably receives some of the incident radiation as a result of the inherent scattering properties of the solutions themselves, or if dust or other solids are present. Filters are used to eliminate this residual scatter. The primary filter selects short wavelength radiation capable of causing excitation of the test substance, while the secondary filter is normally a sharp cut-off filter that allows the longer wavelength fluorescence to be transmitted but blocks the scattered excitated radiation.

Most fluorimeters use photomultiplier tubes as detectors; many types are available, each having special characteristics with respect to spectral region of maximum sensitivity, gain, and electrical noise. After amplification of the photocurrent its value is read visually on a measuring device or recorded.

A fluorescence spectrophotometer differs from a filter fluorimeter in that filters are replaced by monochromators, of either the prism or the grating type. For analytical purposes, the fluorescence spectrophotometer is superior to the filter fluorimeter in wavelength selectivity, flexibility, and convenience.

Many radiation sources are used in fluorimeters and fluorescence spectrophotometers. Mercury lamps are relatively stable and emit energy mainly at discrete wavelengths. Tungsten lamps provide an energy continuum in the visible region. The high pressure xenon arc lamp is often used in fluorescence spectrophotometers because it has an energy continuum extending from the ultraviolet into the infrared.

In fluorescence spectrophotometers the monochromators are equipped with slits. A narrow slit provides high resolution and spectral purity, while a large slit sacrifices these for high intensity. Choice of slit-width is determined by the wavelength separation between incident and emitted radiation, as well as by the degree of sensitivity needed.

The cells used in fluorescence measurements may be rectangular cells similar to those used in absorption spectrophotometers, except that they are polished on all 4 vertical sides and on the bottom, or cells in the shape of round tubes with flat polished bottoms may be used. A convenient size is 2–3 ml, but some instruments can be fitted with small cells holding 0.1–0.3 ml, or with a capillary holder requiring even less solution.

Standardization

Fluorimeters and fluorescence spectrophotometers should be standardized daily with a stable fluorophore to assure proper reproducibility of response. The changes are due to instrumental variables such as differences in lamp intensity and photomultiplier sensitivity. The fluorophore may be a pure specimen of the fluorescent substance under test or another readily purified fluorescent substance with absorption and fluorescence bands similar to those of the test substance. Quinine in dilute sulfuric acid is often a convenient fluorophore for

blue fluorescence, sodium fluorescein for green fluorescence, and rhodamine for red fluorescence.

Calibration of the wavelength scale

The wavelength scale of the fluorescence spectrophotometer should be periodically calibrated.

Preparation of solution

The solvent used for the measurement should be properly selected. The solvent itself, its purity and its pH may markedly affect the intensity and spectral distribution of fluorescence.

Test solutions prepared for fluorescence spectrophotometry are usually 10 times to 100 times less concentrated than those used in absorption spectrophotometry. In analytical applications, it is necessary that the fluorescence intensity be linearly related to the concentration in the range used for measurements; but if a solution is too concentrated, a significant part of the incident light is absorbed by the substance near the cell surface, thus resulting in a reduction of the light reaching the centre. That is, the substance itself acts as an "inner filter". Fortunately, fluorescence spectrophotometry is inherently a very sensitive technique, and concentrations of 10^{-5}–10^{-7} mol/l are frequently used.

Owing to the usually very narrow range within which the fluorescence is proportional to the concentration of the fluorescent substance, the ratio $(c–d)/(a–b)$, where a is the fluorescence intensity read for the reference substance, b the reading for the corresponding blank, c the fluorescence intensity read for the test substance, and d the reading for the corresponding blank, should be not less than 0.40 and not more than 2.50. It is then necessary to make a working curve of fluorescence intensity corrected for a solvent blank versus concentration.

Measurement technique

Fluorescence measurements are sensitive to the presence of solid particles in the test solution. Such impurities may reduce the intensity of the exciting beam or give misleadingly high readings because of multiple reflections in the cell. It is, therefore, wise to eliminate solid particles by centrifugation; filtration may also be used, but some filter-papers may contain fluorescent impurities.

Oxygen dissolved in the solvent has a strong quenching effect. The intensity of fluorescence is, therefore, increased by use of a degassing procedure, such as bubbling nitrogen or another inert gas through the test solution.

Temperature regulation is often important in fluorescence spectrophotometry. For some substances, fluorescence efficiency may be reduced by as much as 1–2% per degree of temperature rise. In such cases, if maximum precision is desired, temperature-controlled sample cells are essential. For routine analysis, it may be sufficient to make measurements rapidly enough so that the test solution does not heat up appreciably from exposure to the intense light source.

Many fluorescent compounds are light-sensitive. Exposed in a fluorescence spectrophotometer, they may be photodegraded into more or less fluorescent products. Such effects may be detected by observing the detector response in relation to time, and may be reduced by attenuating the light source with filters or screens.

1.10 Turbidimetry and nephelometry

Turbidimetry is the measurement of the degree of attenuation of a radiant beam incident on particles suspended in a medium, the measurement being made in the directly transmitted beam. It may be measured with a standard photoelectric filter photometer or spectrophotometer with illumination at an appropriate wavelength.

Nephelometry is the measurement of the light scattered by suspended particles, the measurement usually being made perpendicularly to the incident beam.

Turbidimetry or nephelometry may be useful for the measurement of precipitates formed by the interaction of very dilute solutions of reagents, or other particulate matter, such as suspensions of bacterial cells. In order that consistent results may be achieved, all variables must be carefully controlled. Problems due to birefringence may be encountered, particularly with bacterial cells. Where proper control is possible, extremely dilute suspensions may be measured.

Terms

Transmittance (T) – The ratio of the radiant flux transmitted by the test substance to that of the incident radiant flux. Terms formerly used include transmittancy and transmission.

Turbidance (S) – A measure of the light-scattering effect of suspended particles.

Turbidity (τ) – In light-scattering measurements, the turbidity is the measure of the decrease in incident beam intensity per unit length of a given suspension.

Apparatus

Turbidity may be measured with a standard photoelectric filter photometer or spectrophotometer, preferably with illumination in the red-orange region of the spectrum (for example, by using a blue filter).

Nephelometric measurements require an instrument with a photocell placed so as to receive scattered rather than transmitted light; as this geometry applies also to fluorimeters in general, the latter can be used as nephelometers by proper selection of filters.

Instrumental measurement

For instrumental measurement it is advisable to ensure that settling of the particles being measured will be negligible. This is usually accomplished by including a protective colloid in the liquid suspending medium. It is important that results

be interpreted by comparison of readings with those representing known concentrations of suspended matter, produced under exactly the same conditions.

Visual comparison
Carry out turbidity comparison in tubes that are matched as closely as possible in internal diameter and in all other respects. Flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter are suitable. For turbidity comparison the tubes should be viewed horizontally, against a dark background, with the aid of a light source directed from the sides of the tubes.

1.11 Colour of liquids

The test for colour of liquids is carried out by comparing the test solution prepared as specified in the monograph with a standard colour solution indicated in the monograph. The composition of the standard colour solution is selected depending on the hue and intensity of the colour of the test solution corresponding to the limits permitted in the specifications.

Recommended procedure

Unless otherwise specified in the monograph, carry out the comparison in flat-bottomed tubes of transparent glass that are matched as closely as possible in internal diameter and in all other respects (tubes of about 16 mm internal diameter are suitable). Use 10 ml of the test solution and 10 ml of the standard colour solution; the depth of liquid should be about 50 mm. The colour of the test solution is not more intense than the standard colour when viewed down the vertical axis of the tubes in diffused light against a white background.

Stock colour standard solutions

Yellow stock standard TS
To 9.5 ml of cobalt colour TS, add 1.9 ml of copper colour TS, 10.7 ml of dichromate colour TS, 4.0 ml of iron colour TS, dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Red stock standard TS
To 40.5 ml of cobalt colour TS, add 6.1 ml of copper colour TS, 6.3 ml of dichromate colour TS, 12.0 ml of iron colour TS, dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Green stock standard TS
To 3.5 ml of cobalt colour TS, add 20.1 ml of copper colour TS, 10.4 ml of dichromate colour TS, 4.0 ml of iron colour TS, dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Brown stock standard TS
To 35.0 ml of cobalt colour TS, add 17.0 ml of copper colour TS, 8.0 ml of dichromate colour TS, dilute to 100.0 ml with iron colour TS, and mix.

Standard colour solutions

The standard colour solution is prepared by suitably diluting the stock standard solutions (yellow, red, green, and brown stock standard TS) with sulfuric acid (~10 g/l) TS. The designation of the standard colour solution is composed of two letters indicating the stock standard solution (Yw for yellow, Rd for red, Gn for green, and Bn for brown) and of a number describing the dilution, as given below:

Dilution number for standard colour solutions	Stock standard solution (ml)	Sulfuric acid (~10g/l) TS (ml)
0	0.78	99.22
1	1.56	98.44
2	3.12	96.88
3	6.25	93.75
4	12.50	87.50
5	25.00	75.00
6	50.00	50.00
7	100.00	–

Standard colour solution numbers 4–7 may be stored in sealed glass containers, protected from sunlight but the more dilute standard colour solutions should be prepared as required.

1.12 Powder fineness and sieves

A. Powders

The degree of coarseness or fineness of a powder is differentiated by the nominal aperture size of the mesh of the sieve through which the powder is able to pass, expressed in µm.

The following terms are used in the description of powders:

Coarse powder (2000/355). A powder of which all the particles pass through a No. 2000 sieve, and not more than 40% through a No. 355 sieve.

Moderately coarse powder (710/250). A powder of which all the particles pass through a No. 710 sieve, and not more than 40% through a No. 250 sieve.

Moderately fine powder (355/180). A powder of which all the particles pass through a No. 355 sieve, and not more than 40% through a No. 180 sieve.

Fine powder (180). A powder of which all the particles pass through a No. 180 sieve.

Very fine powder (125). A powder of which all the particles pass through a No. 125 sieve.

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder should pass through the sieve distinguished by that number.

B. Sieves

The wire sieves used in sifting powdered drugs are distinguished by numbers, which indicate the nominal aperture size expressed in μm.

The sieves are made of wires of uniform circular cross-section, in accordance with the specifications given in Table 2.

The nominal size of aperture of wire mesh sieves has been selected principally from among those recommended in the ISO standard 565-1972.

1.13 Determination of pH

A value characteristic of an aqueous solution is its pH value, which represents conventionally its acidity or alkalinity.

The pH of a solution is the negative logarithm of the hydrogen ion activity, which may be measured potentiometrically. Formerly, pH was regarded as the negative logarithm of the hydrogen ion concentration. As it is known that not all hydrogen ions are necessarily equally active, this concentration may be different from the hydrogen ion activity. However, if the activity coefficient is

Table 2. Wire mesh sieves

Number of sieve (μm)	Nominal size of aperture (mm)	Nominal diameter of wire (mm)	Approximate screening area (%)
2000	2.00	0.90	48
710	0.710	0.450	37
500	0.500	0.315	38
355	0.355	0.224	38
250	0.250	0.160	37
212	0.212	0.140	36
180	0.180	0.125	35
150	0.150	0.100	36
125	0.125	0.090	34
90	0.090	0.063	35
75	0.075	0.050	36
45	0.045	0.032	34

close to 1, as is true in dilute solutions, the values of hydrogen ion activity and hydrogen ion concentration become nearly identical.

The determination of the pH value is carried out by measurement of the potential difference between electrodes immersed in standard and test solutions. The standard solutions used are assigned a definite pH value by convention.

In the measurement of pH, glass electrode finds wide applicability as it shows an immediate response to rapid changes of hydrogen ion concentrations even in poorly buffered solutions. Since the mechanism of this electrode involves no electron exchange it is the only electrode sensitive to hydrogen ions that is not disturbed by oxidizing or reducing agents.

The pH values of solutions or suspensions that are only partially aqueous and that can be considered only as "apparent pH values" can also be measured by using the proper electrode and by suitably standardizing the pH-meter.

As pH values are dependent on temperature, the measurements are carried out at selected constant temperatures.

Solutions used in determinations of pH are prepared with carbon-dioxide-free water R.

pH Scale

The difference in pH between two solutions, *X* and *S*, at the same temperature may be defined operationally as follows:

The electromotive force E_x, of the cell

Pt|H_2| solution *X* |3.5 mol/l KCl| reference electrode

and the electromotive force, E_s, of the cell

Pt|H_2| solution *S* |3.5 mol/l KCl| reference electrode

are measured, both cells being at the same temperature throughout and the reference electrodes and bridge solutions being identical in the two cells.

The pH of solution *X*, denoted by pH (*X*), is then related to the pH of solution *S*, denoted by pH (*S*), by the equation:

$$pH(X) = pH(S) + \frac{E_X - E_S}{2.3026\,RT/F}$$

where *R* denotes the gas constant, *T* the thermodynamic temperature (in K), and *F* the Faraday constant. Thus defined, the quantity pH is a dimensionless number.

Numerical values of the factor 2.3026 *RT/F* at several temperatures are given below:

Temperature (in °C)	2.3026 RT/F (mV)
10	56.18
15	57.17
20	58.17
25	59.16
30	60.15

Potentiometric determination of pH

For the practical determination of pH, a potentiometric method is usually employed. When glass electrodes are used they should be stored in a suitable liquid, usually water.

It is often found more convenient to measure pH by means of a glass electrode instead of the hydrogen electrode. In some solutions, especially those containing oxidizing agents, the glass electrode can be used when the hydrogen electrode cannot. The accuracy and reproducibility of ±0.005 usually obtainable with the hydrogen electrode are, however, rarely obtainable with the glass electrode, and never outside the pH range 2–10.

When the glass electrode is used, the best accuracy is obtained by assuming that over a short range of pH there is a linear relation between pH and measured electromotive force, but that the proportionality factor relating them is not necessarily exactly 2.3026 *RF/T*. This method of using the glass electrode necessitates calibration by means of two solutions of known pH, near to, and preferably bracketing, the pH to be measured. There are at present various suitable solutions, the pH values of which are reliably known to an accuracy of ±0.005 (see Table 3).

Calibration of apparatus

The apparatus is calibrated with standard buffer solutions to check the linearity of the response of the electrode at different pH values and to detect a faulty

Table 3. Standard buffer solutions and their pH values

Standard buffer solutions	pH values at various temperatures				
	20 °C	25 °C	30 °C	35 °C	40 °C
Potassium tetraoxalate standard TS	1.675	1.679	1.683	1.688	1.694
Potassium hydrogen tartrate standard TS	–	3.557	3.552	3.549	3.547
Potassium hydrogen phthalate standard TS	4.002	4.008	4.015	4.024	4.035
Phosphate standard buffer, pH 6.8, TS	6.881	6.865	6.853	6.844	6.838
Phosphate standard buffer, pH 7.4, TS	7.429	7.413	7.400	7.389	7.380
Sodium tetraborate standard TS	9.225	9.180	9.139	9.102	9.068
Sodium carbonate standard TS	10.062	10.012	9.966	9.925	9.889

glass electrode. The standardization of the apparatus with only a single solution may be completely erroneous and therefore at least two standard buffer solutions should be used for calibration. The presence of a faulty electrode will be detected by failure to obtain a reasonably correct value (±0.04 unit) for the pH of the second standard solution when the apparatus has been standardized in terms of the first standard. A cracked electrode will yield pH values that are essentially the same for both solutions. If the difference between the known and the observed pH values for the second solution exceeds ±0.04, another glass electrode should be substituted. If the difference persists, fresh standard solutions should be prepared.

Recommended procedure

After the apparatus has been calibrated, thoroughly wash the electrodes and the cup. Fill the cup with a portion of the solution to be tested and obtain a preliminary value for the pH. In general, this value will drift and is regarded as an approximation. Subsequent readings taken on additional portions of the same solution will yield successively more constant pH values. In the case of solutions that are well buffered, 3 portions may be sufficient to yield pH values that are reproducible to ±0.04 unit and that show drifts of less than ±0.04 unit in 1 or 2 minutes. In the case of very dilute or unbuffered solutions, as many as 6 portions of the test solution may be required, and the pH values may continue to drift and be reproducible to only ±0.05 unit.

If a precision greater than 0.1 pH unit is desired, the temperature of the standard solutions, the glass and calomel electrodes, and the test solutions must be within 2 °C of one another, and the electrodes, standard solutions, test solutions, and wash water must be kept at the temperature of measurement for at least 2 hours prior to making the measurement in order to reduce to a negligible value the effects of thermal or electrical hysteresis of the electrodes.

Standard buffer solutions

Standard buffer solutions are used in the determination of pH values. They are prepared with carbon-dioxide-free water R. They should be stored in bottles of chemically resistant glass or in bottles made of polyethylene.

Unless otherwise specified, standard buffer solutions should not be used later than 3 months after preparation. If growth of microorganisms starts in the solutions they should immediately be discarded and the bottles thoroughly cleaned and sterilized before refilling.

1.14 Chromatography

Chromatographic processes involve, in general, the distribution of a solute between two phases, one of which is the mobile phase and the other the stationary phase. The stationary phase may act by adsorption, by partition (where, for example, a liquid that is immiscible with the mobile phase may be coated

on the surface of a solid support), by ion exchange, or by gel permeation. In practice, the chromatographic process in many pharmaceutical applications may be a complex synthesis of several physical phenomena; certainly many chromatographic procedures that are referred to as partition methods may be significantly influenced by adsorption effects.

Chromatography is thus simply a method of separation and of itself does not permit either identification or quantitative measurement. It is the combination of chromatography with appropriate methods of detection and measurement that results in the loosely but conveniently termed "chromatographic method of analysis".

For convenience the types of chromatography that are useful in pharmaceutical analysis may be considered as falling into three broad groups. In the planar methods, chromatography is effected by allowing the mobile phase to flow over and through a layer of the adsorbent (paper and thin-layer chromatography). In column methods, the adsorbent is packed into a column, which may be of the traditional open type or may be closed and designed to withstand considerable pressures so that the mobile phase may be pumped through the column at high speed (high-pressure liquid chromatography – sometimes known as high-performance or high-speed liquid chromatography). Gas chromatography is a special case of column chromatography in that the mobile phase is a gas rather than a liquid and the solutes must be volatile or rendered so by elevated temperatures and/or by conversion to volatile derivatives.

No one method of chromatography is adequate for all purposes, since each has its advantages and disadvantages. Most of the planar methods are simple and effective and require inexpensive equipment (although some complex accessories are available); these methods are valuable for identification and screening purposes but are less suitable for precise quantitative applications. Older column methods may be used without great expense but are often time-consuming and tedious to carry out. High-pressure liquid and gas chromatographic methods need specialized apparatus but may afford rapid and effective separations that are suitable for precise quantitative measurement of components. Such methods are particularly valuable for the determination of small amounts of impurities. Limitations are currently imposed on the value of high-pressure liquid chromatography by the lack of universally applicable methods of detection and on that of gas-liquid chromatography by the non-volatility or thermal instability of many compounds.

1.14.1 Thin-layer chromatography

In thin-layer chromatography the adsorbent is a thin, uniform layer (usually about 0.24 mm thick) of a dry, finely powdered material applied to a suitable support, such as a glass plate or an aluminium or plastic foil. The mobile phase is allowed to move across the surface of the plate (usually by capillary action) and the chromatographic process may depend upon adsorption, partition, or a combination of both, depending on the adsorbent, its treatment, and the nature

of the solvents used. During the chromatographic procedure the plate is contained in a chromatographic chamber (usually made of glass to permit observation of the movement of the mobile phase up the plate), which is usually saturated with the solvent vapour. Solid supports frequently used are silica gel, kieselguhr, alumina, and cellulose; to these may be added suitable substances, for example, calcium sulfate to promote adhesion to the support. The prepared layer may be impregnated with buffering materials to afford acidic, neutral, or basic layers, or with other material, such as silver nitrate, designed to modify its properties. In certain cases the layer may consist of an ion-exchange material. This wide range of possible layers, used in conjunction with different solvent systems allows an almost infinite variation of separating power that makes thin-layer chromatography such a useful technique in pharmaceutical analysis.

As an adjunct to identification, thin-layer chromatography may be used by comparing the behaviour of the material to be identified with that of a standard substance, usually an authentic specimen of the substance being examined. If the two substances move identical distances during the chromatographic process and if the two substances, when mixed together and then subjected to chromatography, move as a single substance, it may be presumed that the two substances are identical. This presumption may be strengthened by repeating the procedure using a different system of chromatography; in general, if two substances behave identically in as many as three fundamentally different systems the presumption of identity becomes very strong.

For identification purposes it convenient to define the relative distance that the unknown material moves in relation to the distance moved either by the solvent front or by a standard reference material. On a developed chromatogram, the ratio of the distance travelled on the adsorbent by a given compound to that travelled by the leading edge oft he solvent (or mobile phase), both measured from the point of application of the test substance, is referred to as the R_f value of the substance in the given chromatographic system. The ratio of the distances moved by the compound and a stated reference substance is referred to as the R_r value. In practice R_f values may vary considerably according to the exact experimental conditions so that the R_r value determined against the reference substance subjected to chromatography on the same surface gives a more reliable numerical value. Even more reliable, however, is comparison with an authentic specimen as described above and this is the procedure usually used for pharmacopoeial purposes.

To determine the position of a colourless substance on a developed chromatogram it is usually necessary to treat the chromatogram with a reagent that will either char the separated substances or convert them to coloured or fluorescent derivatives. A convenient alternative that is frequently applicable is to carry out the chromatography on a surface impregnated with a substance that fluoresces strongly when examined under short-wave ultraviolet light. Areas of the plate occupied by substances absorbing at the same wavelengthshow as dark spots on the fluorescent background. In special cases, other means of

recognition may be used, for example, the detection of radioactivity where labelled compounds are being separated or a microbiological response where antibiotics are concerned.

The most valuable use of thin-layer chromatography in pharmaceutical work is to provide a means of assessing low levels of impurities in medicinal substances. For this purpose the substance is applied to the chromatographic surface and, after chromatography, any secondary spots to be seen in the chromatogram after appropriate visualization are compared for size and intensity with those of low loadings of expected impurities that have simultaneously been subjected to chromatography on the same plate. Such a procedure requires that the expected impurities be available and in certain monographs the use of authentic specimens of impurities is called for. Frequently, such impurities are not available and in such cases it is often possible to compare secondary spots arising from trace impurities with the spot obtained by carrying out chromatography on the same plate using an appropriately low loading of the substance being examined. This expedient is not always possible since impurities and the substance being examined may respond in different ways to the method of revelation used, but it often provides an acceptable criterion by which the level of impurity in the substance may be judged. A third procedure that is sometimes advocated is to apply such an amount of the substance being examined that, after chromatography, no secondary spots will appear if the sample is acceptably pure. This is the least satisfactory of the three methods since ability to see a secondary spot is a subjective matter and because the intensity of spots on a chromatogram may vary considerably from one occasion to another depending on the exact conditions of chromatography.

For quantitative procedures the spot may be removed from the plate, the substance eluted with a suitable solvent and then determined by a sufficiently sensitive method, such as a spectrophotometric measurement, either directly or after a chemical reaction. In certain cases, quantitation can also be achieved by measurement of the spot intensity with the aid of a scanning densitometer and subsequent comparison of the intensity with the intensities obtained from standard amounts of the same substance similarly treated.

Separations effected by thin-layer chromatography may sometimes be improved by multiple development (when the chromatogram is allowed to dry and then subjected again to the same system of chromatography), by continuous development (when the mobile phase is allowed to evaporate continuously from the upper end of the adsorbent surface), or by two-dimensional chromatography (when the chromatographic plate is allowed to dry, turned at right angles, and then subjected to further chromatography, frequently in a different solvent system from that used for the initial chromatography). Caution should be exercised in interpreting the results of chromatograms where such intermediate drying processes are used, however, since decomposition, such as oxidation, of the substance being chromatographed may occur on the plate. The process of two-dimensional chromatography is especially valuable in judging whether any chemical change is taking place during the development process.

If a mixture of substances is developed first in one direction and then at right angles with the same solvent the separated substances will lie in a diagonal line across the plate if no artefacts are being produced.

In thin-layer chromatography the adsorbent is usually spread in a thin even layer on a support plate. This may be undertaken in the analytical laboratory but it is also possible and convenient to obtain commercially prepared chromatographic surfaces that are attached to glass or to plastic or metal foil. Unfortunately, so sensitive may the chromatographic process be to minor changes in conditions that these various commercially available materials are not always interchangeable one with another or with an apparently similar laboratory-coated plate. A factory-coated silica gel plate prepared by a given manufacturer may give different separation characteristics from a laboratory-coated plate prepared using the same manufacturer's coating substance, and instances exist where a perfectly satisfactory method of separation devised using laboratory-prepared plates fails when using precoated plates and vice versa. Great caution must, therefore, be exercised when changing from one type of chromatographic plate to another and the suitability of a given type of plate should always be assessed before reliance is placed upon it.

The chromatographic chamber in which chromatography takes place should be protected from light if it is suspected that the materials to be examined may be unstable in light. In any case, the chromatographic chamber should always be in a position where the direct rays of the sun cannot fall on it since the rays may be refracted to different degrees owing to imperfections in the glass walls of the chamber. This may give rise to areas of elevated temperature on the chromatographic plate and result in erratic flow of the mobile phase.

Recommended procedure

The method given below assumes the use of a laboratory-prepared chromatographic plate but a precoated plate, activated if necessary, may be used provided that it has been shown to be suitable for the particular application.

The equipment consists of:

- a device for spreading on plates a uniform layer of coating substance of the desired thickness;
- plates 200 mm long and wide enough to accommodate the required number of solutions to be examined and the reference solutions;
- a chromatographic chamber of transparent material, usually glass, with a tightly fitting lid, of a size suitable for the plates used.

Prepare a slurry of the coating substance and, using the spreading device, coat the carefully cleaned plates with a layer about 0.25 mm thick, unless otherwise specified in the monograph. Allow the coated plates to dry in air and heat to activate, unless otherwise specified in the monograph, at 110 °C for 30 minutes, then allow to cool. If the plates are not to be used immediately, store them in a desiccator containing silica gel, desiccant, R. Remove a narrow strip (2–5 mm) of the coating substance from the vertical sides of the plate.

Unless otherwise specified in the monograph, work under saturated chamber conditions. To achieve such conditions, line the chromatographic chamber with filter-paper and pour into the chamber a sufficient quantity of the mobile phase to saturate the filter-paper and form a layer about 5 mm deep. Close the chamber and allow to stand for at least 1 hour at room temperature.

Method

All operations during which the plate is exposed to the air should preferably be carried out at a relative humidity of 50–60%. Apply the volume of the solution as specified in the monograph as a compact spot, preferably not more than 4 mm in diameter. Application may be made using a micropipette, a syringe, or other suitable means. The spot should be placed about 1.5 cm from the lower edge and not less than 2 cm from the vertical sides of the plate. Where more than one chromatogram is run on the same plate, the spots should be placed not less than 1.5 cm apart and form a line parallel with the lower edge of the plate. When the solvent has evaporated, place the plate in the chromatographic chamber, ensuring that the plate is as nearly vertical as possible and that the starting points are above the level of the mobile phase. Close the chamber and maintain it at a constant temperature. Allow the mobile phase to ascend, usually 10–15 cm, remove the plate, mark the position of the solvent front and dry as specified in the monograph.

1.14.2 Paper chromatography

In paper chromatography the stationary phase is a sheet of paper of suitable texture and thickness, which may sometimes be impregnated with a liquid phase that is immiscible with the mobile phase.

Chromatographic separations on paper are usually considerably slower than on thin-layer plates and the method is, in general, not so versatile as thin-layer chromatography since the degree of variation of the stationary phase is much more restricted. Neither is it possible to use the variety of corrosive detection reagents that is commonly employed when the adsorbent is an inorganic material coated on a glass plate. Nevertheless, paper chromatography is still a useful technique and certain very effective separations that were originally devised using paper have never been successfully transferred to the thin-layer plate. For semi-quantitative and quantitative work it is considerably easier and more effective to cut out a required area of paper and to elute a separated component than it is to remove completely the powder layer for elution, as is necessary with thin-layer chromatography.

The concepts of R_f and R_s values referred to in the discussion on thin-layer chromatography apply equally well to paper chromatography. Because of the nature of the adsorbent it is possible to carry out paper chromatography in either a descending or an ascending mode.

Recommended procedure

Descending paper chromatography

The apparatus consists of a glass chamber of suitable dimensions to accommodate the chromatographic paper used, ground at the top to take a closely fitting glass lid. The lid has a central hole about 1.5 cm in diameter closed by a heavy glass rod or a stopper. In the upper part of the chamber is suspended a solvent trough with a device, usually a glass rod, for holding the chromatographic paper. On either side of the trough, parallel to and slightly above its upper edges, are two glass guide rods to support the paper in such a manner that no part of it is in contact with the walls of the chamber. The chromatographic paper consists of suitable filter-paper, cut into strips of sufficient length, and of any convenient width between 2.5 cm and the length of the trough; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method Place in the bottom of the chromatographic chamber a layer 2–3 cm deep of the stationary phase specified in the monograph. Close the chamber, and allow to stand for 24 hours at constant temperature. All operations during which the paper is exposed to the air should preferably be carried out at a relative humidity of about 50%. Maintain the chamber under these conditions throughout the subsequent procedure. Draw a fine pencil line horizontally across the paper at such a distance from one end that when this end is secured in the solvent trough and the remainder of the paper is hanging freely over the guide rod the line is a few centimetres below the guide rod and parallel to it. Using a micropipette, syringe, or other suitable means, apply to a spot on the pencil line the volume of the solution specified in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter, apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper in the chamber, close the lid, and allow to stand for 90 minutes. Introduce into the solvent trough, through the hole in the lid, a sufficient quantity of the mobile phase specified in the monograph, close the chamber and allow development to proceed for the prescribed distance or time. Remove the paper from the chromatographic chamber and allow to dry in air. The paper should be protected from bright light during the development and drying processes.

Ascending paper chromatography

The apparatus consists of a glass chamber of suitable dimensions to accommodate the chromatographic paper used, ground at the top to take a closely fitting glass lid. In the top of the chamber is a device that suspends the chromatographic paper and is capable of being lowered without opening the chamber. In the bottom of the chamber is a dish to contain the mobile phase into which the paper may be lowered. The chromatographic paper consists of

suitable filter-paper, cut into strips of sufficient length and not less than 2.5 cm wide; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method Place in the dish a layer 2–3 cm deep of the mobile phase specified in the monograph. If specified in the monograph, pour the stationary phase between the walls of the chamber and the dish. Close the chromatographic chamber and allow to stand for 24 hours at constant temperature. Maintain the chamber at this temperature throughout the subsequent procedure. All operations during which the paper is exposed to the air should preferably be carried out at a relative humidity of about 50%. Draw a fine pencil line horizontally across the paper 3 cm from one end. Using a micropipette, syringe, or other suitable means, apply to a spot on the pencil line the volume of the solution specified in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper into the chamber, close the lid, and allow to stand for 90 minutes. Lower the paper into the mobile phase specified in the monograph, and allow development to proceed for the prescribed distance or time. Remove the paper from the chamber and allow to dry in air. The paper should be protected from bright light during the development and drying processes.

1.14.3 Column chromatography

In *adsorption column chromatography* the solid support (e.g. activated alumina, powdered cellulose, silicic acid, or kieselguhr) is packed as a dry solid or as a slurry into a tube (glass, plastic, or other suitable material) having a restricted orifice (usually protected by a sintered glass disc) for efflux of the mobile phase. A solution of the material to be subjected to chromatography is added to the top of the column and allowed to flow into the adsorbent; the solvent that constitutes the mobile phase is then introduced in the top of the column and allowed to flow downwards either by gravity or by application of positive pressure; during this procedure care should be taken to ensure that the top of the column does not become dry. The effluent solution (known as the eluate) is monitored in either a continuous fashion (for example, with a flow-through ultraviolet absorption cell) or a stepwise fashion (for example, by collection of fractions at intervals determined by time or by volume or weight of eluate and subsequent examination of each fraction for the separated component). The need to examine individually many fractions in order to obtain a completely quantitative assessment of a substance has resulted in a decline in the use of classical column chromatographic procedures in recent years; where they do continue to be used, there is a natural tendency to favour methods of detection and determination that are readily adaptable to automated processes.

In *partition column chromatography,* a liquid stationary phase, which should be substantially immiscible with the mobile phase, is adsorbed to the surface of the solid adsorbent. Chromatography is carried out as described for adsorption column chromatography, the mobile phase being saturated with the stationary phase before it is used for elution. Usually the solid adsorbent in partition chromatography is polar and the adsorbed stationary phase is also polar with respect to the mobile phase. The most widely used adsorbent in this connexion is a siliceous earth having an appropriate particle size to permit ready flow of the mobile phase. In certain cases *reverse-phase partition chromatography* is useful; in this case the polar adsorbent is rendered non-polar by silanizing or other means, such as treatment with paraffins, and the adsorbed stationary phase is less polar than the mobile phase.

In these partition systems the degree of partition of a compound is governed by its distribution coefficient between the two liquid phases and, in the case of compounds that dissociate, by the pH of the more polar of the two phases. Selective elution of components of a mixture can often be achieved by successive changes in the mobile phase or by changing the pH of the stationary phase by using a mobile phase consisting of a solution of an appropriate acid or base in an organic solvent.

Ion-exchange chromatography may be considered a case of chromatography where the solid phase contains an ion-exchange material, usually called ion-exchange resin.

Ion-exchange is defined as the reversible interchange between the ion present in the solution and the counter-ion of the resinous polymer, modified cellulose, or bonded silica gel support; it may be exemplified for the H^+/Na^+ exchange of a strongly acidic cation-exchange resin as:

$$RSO_3H + Na^+ \rightleftharpoons RSO_3Na + H^+$$

and for a Cl^-/OH^- strongly basic anion-exchange resin as:

$$R'N(CH_3)_3OH + Cl^- \rightleftharpoons R'N(CH_3)_3Cl + OH^-$$

The selection of strong or weak resins, of either type, will largely depend on the pH at which the exchange is to be carried out and on the types of cation or anion that are to be exchanged. However, the strongly acidic and basic exchange resins will serve in most analytical applications. Their specific capacity may vary from 2 to 5 millimoles per gram (dry basis). In practice, a large (200–300%) excess of resin is used over the calculated stoichiometric requirement.

The laws governing the exchange reaction are complex, being in part described by mass action, ionic charge, and activity relationships. The selectivity coefficient is used to indicate the preference of the ion-exchange resin for the uptake of 2 (or more) ions from solution. Generally speaking, the resin will take up divalent (or higher) ions in preference to monovalent ions, and in the case of a choice between ions of the same valence, the resin will take up the heavier ion preferentially.

Treatment of the ion-exchange resin and preparation of the column. Usually the ion-exchange resin is immersed in water and allowed to swell for 24 hours; it is then packed into a suitable column and, in the case of an anion-exchange resin, converted to the basic form by passing sodium hydroxide (~80 g/l) TS through the column at a rate of about 3 ml per minute until the effluent is free of chloride, followed by carbon-dioxide-free water, R to remove alkalinity. In the case of a cation-exchange resin, conversion to the acidic form is achieved by passing hydrochloric acid (~70 g/l) TS through the column, followed by carbon-dioxide-free water R until the washings are neutral.

The prepared column is used in a similar manner to that described for adsorption column chromatography except that there is usually no need to monitor the effluent; according to the type of resin chosen and the type of material being determined the volume of effluent detailed in the particular application is collected and titrated with acid or base as appropriate, using a suitable indicator.

After the determination has been completed, the ion-exchange column may be regenerated by washing either with sodium hydroxide (~80 g/l) TS, for an anion-exchange column, or hydrochloric acid (~70 g/l) TS, for a cation-exchange column, followed by water until a neutral reaction is obtained.

1.14.4 High performance liquid chromatography

Introduction

High-performance liquid chromatography (HPLC) is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases.

HPLC can be used to assess the purity and/or determine the content of many pharmaceutical substances. It can also be used to determine enantiomeric composition, using suitably modified mobile phases or chiral stationary phases. Individual separation mechanisms of adsorption, partition, ion exchange and size exclusion rarely occur in isolation, since several principles act to a certain degree simultaneously.

Apparatus

The apparatus consists of a pumping system, an injector, a chromatographic column, stationary and mobile phases, connecting tubing and fittings, a detector, and a data collection device (computer, integrator or recorder).

Pumping system

HPLC pumping systems are required to deliver metered amounts of mobile phase at a constant flow rate. Pumping systems that deliver solvent from one or more reservoirs are available. Pressure fluctuations should be minimized, e.g.

by passing the pressurized solvent through a pulse-dampening device. Tubing and connections should be capable of withstanding the pressures developed by the pumping system. Many HPLC pumps are fitted with a facility for "bleeding" the system of entrapped air bubbles.

Modern computer- or microprocessor-controlled pumping systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or varying (gradient elution) composition, according to a defined programme. In the case of gradient elution, solvent mixing can be achieved on either the low or high-pressure side of the pump(s). Depending on the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (6000 psi) can be generated during routine analysis.

Injector

The sample solution is usually introduced into the flowing mobile phase at or near the head of the column using an injection system based on an injection valve design which can operate at high pressure. Such an injection system has a fixed-loop or a variable volume device which can be operated manually or by an auto-sampler. Partial filling of loops may lead to poorer injection volume precision.

Chromatographic column

Columns are usually made of polished stainless steel, are between 50 and 300 mm long, and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 5–10 µm. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally, the temperature of the mobile phase and the column should be kept constant during an analysis. Most separations are performed at ambient temperature but columns may be heated using, for instance, a water-bath, a heating block or a column oven in order to achieve better efficiency. Normally, columns should not be heated above 60 °C because of the potential for stationary phase degradation or changes occurring to the composition of the mobile phase.

Stationary phases

Separation of pharmaceuticals is usually achieved by partition of compounds in the test solution between the mobile and the stationary phases. HPLC systems consisting of polar stationary phases and nonpolar mobile phases are described as normal-phase chromatography; those with nonpolar stationary phases and polar mobile phases are called reversed-phase chromatography.

There are many types of stationary phases used in HPLC including:

— unmodified silica, alumina, or porous graphite, used in normal-phase chromatography, where separation is based on differences in adsorption;
— a variety of chemically modified supports prepared from polymers, silica, or porous graphite, used in reverse-phase HPLC, where separation is

based principally on partition of the molecules between the mobile phase and the stationary phase;
— resins or polymers with acid or basic groups, used in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase;
— porous silica or polymers, used in size-exclusion chromatography, where separation is based on the relative molecular mass of the molecules.

Most separations are based on partition mechanisms using chemically modified silica as the stationary phase and polar solvents as the mobile phase (reverse-phase HPLC). The surface of the support, e.g. the silanol groups of silica, is reacted with various silane reagents to produce covalently bonded silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Commonly used bonded phases are shown below.

Common bonded phases		
octyl	$Si\text{-}(CH_2)_7\text{-}CH_3$	C_8
octadecyl	$Si\text{-}(CH_2)_{17}\text{-}CH_3$	C_{18}
phenyl	$Si\text{-}(CH_2)_3\text{-}C_6H_5$	C_6H_5
cyanopropyl	$Si\text{-}(CH_2)_3\text{-}CN$	CN
aminopropyl	$Si\text{-}(CH_2)_3\text{-}NH_2$	NH_2
diol	$Si\text{-}(CH_2)_3\text{-}OCH(OH)\text{-}CH_2\text{-}OH$	

For the separation of enantiomers, special chemically modified stationary phases (chiral chromatography) are available, e.g. cyclodextrins, albumins, etc.

As a guide, silica-based reverse-phase columns are generally considered to be stable in mobile phases with an apparent pH in the range 2.0–8.0, but the column manufacturer's instructions should be consulted before using the column. Columns containing particles of polymeric materials such as styrene divinylbenzene copolymer are stable over a wider pH range.

Analysis using normal-phase HPLC with unmodified silica, porous graphite or polar chemically modified silica (e.g. cyanopropyl or diol) as the stationary phase and a nonpolar mobile phase is still employed in certain cases.

For analytical separations the particle size of the most commonly used stationary phases varies between 3 µm and 10 µm. The particles may be spherical or irregular, of different porosities and specific surface area. In the case of reversed-phase, the extent of bonding of the stationary phase is expressed as the carbon-loading. Furthermore, stationary phases may be "end-capped", i.e. the number of residual silanol groups is reduced by methylation. These parameters contribute to the chromatographic behaviour of a particular stationary phase. Tailing of peaks, particularly for basic substances, can occur when residual silanol groups are present.

Mobile phases

The choice of mobile phases is based on the desired retention behaviour and the physicochemical properties of the analyte.

For normal-phase HPLC using unmodified stationary phases, lipophilic solvents should be employed. The presence of water in the mobile phase must be avoided as this will reduce the efficiency of the stationary phase. In reverse-phase HPLC aqueous mobile phases, with and without organic modifiers, are used.

The mobile phase should be filtered through suitable membrane-type filters with a porosity of 0.45 µm to remove mechanical particles. Multicomponent mobile phases should be prepared by measuring the required volumes (unless masses are specified) of the individual components, followed by manual or mechanical mixing. Alternatively, the solvents may be delivered by the individual pumps or proportioning valves of the liquid chromatograph and mixed according to the desired proportion. Solvents are normally degassed by sparging with helium or by sonification before pumping to avoid the creation of gas bubbles in the detector cell.

If an ultraviolet detector is employed, the solvents used for the preparation of the mobile phase should be free of stabilizers and transparent at the wavelength of detection. Adjustment of the pH, if necessary, should be made using the aqueous component of the mobile phase and not the mixture. Buffers of high molarity should be avoided in the preparation of mobile phases. If buffers are used, the system should be rinsed with an adequate mixture of water and the organic modifier of the mobile phase to prevent crystallization of salts.

Mobile phases may contain other components, e.g. a counter-ion for ion-pair chromatography or a chiral selector for chiral chromatography using an achiral stationary phase.

Connecting tubing and fittings

The potential efficiency of an analytical column may never be achieved because of the design limitations of injectors and detectors. The connections between injector/column, column/detector, and/or detector/detector may compromise the overall efficiency of the system and any fittings should be of the "zero dead volume" (ZDV) type. It is recommended that minimum lengths of capillary tubing with a maximum internal diameter of 0.25 mm be used for these fittings to minimize band spreading.

Detectors

Ultraviolet/visible (UV/vis) absorption spectrometers are the most commonly used detectors for pharmaceutical analysis. In specific cases, fluorescence spectrophotometers, differential refractometers, electrochemical detectors, light-scattering detectors, mass spectrometers, or other special detectors may be used. Where an analyte possesses a chromophoric group that absorbs UV/vis radiation, the UV/vis detector is the most appropriate because of its sensitiv-

ity and stability. Such a detector is not suitable for detecting analytes with very weak chromophores.

A variant on the UV/vis type of detector, which is becoming increasingly popular because of its ability to furnish detailed spectral information, is the diode array spectrophotometer. This type of detector acquires absorbance data over a certain UV/vis range and can provide chromatograms at multiple, selectable wavelengths, together with spectra for the eluted peaks. In addition, the detector and accompanying computer programmes can be used to assess the spectral homogeneity of peaks, which may provide information on the chromatographic purity of the peaks. This can be especially useful in method development and validation.

Enhanced sensitivity may be achieved in certain cases by using pre-column or post-column derivatization techniques. (These techniques are to be avoided for the control of impurities.)

Data collection devices

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store, and reprocess chromatographic data. The data storage capacity of these devices is usually limited.

Modern data stations are computer based and have a large storage capacity to collect, process, and store data for possible subsequent reprocessing. Analytical reports can often be customized to the needs of the analyst.

Integration of peak areas and the setting of threshold levels are not normally problematic in an assay since the peak of the substance to be analysed should be free of interference. However, in a test for impurities, the selection of the peak area integrator parameters becomes very important, particularly when baseline separations are not always attainable. If baseline separations cannot be obtained, valley-to-valley integration should be employed.

HPLC allows limits to be set for individual impurities and for the sum of impurities, but there is a level at which peaks should not be integrated. This "disregard level" is set in relation to the area of the peak in the chromatogram of the prescribed reference solution and is usually equivalent to 0.05% of the substance being examined.

System suitability

The system suitability test represents an integral part of the method and is used to ensure the adequate performance of the chosen chromatographic system.

Efficiency, capacity factor, resolution factor, and symmetry factor are the parameters that are normally used in assessing the column performance; these terms are defined below. Factors that can affect chromatographic behaviour include mobile phase composition, temperature, ionic strength and apparent pH, flow rate, and column length, and stationary phase characteristics such as porosity, particle size and type, specific surface area, and, in the case of

reversed-phase supports, the type of chemical modification, carbon loading, and end-capping.

Efficiency (N)
The efficiency of a chromatographic column is defined in terms of the number of theoretical plates (N) and can be calculated using the following formula:

$$N = 5.54 \frac{t_R^2}{W_h^2}$$

where t_R = retention time or the baseline distance between the point of injection and the perpendicular dropped from the maximum of the peak of interest.
W_h = the width of the peak of interest determined at half peak height, measured in the same units as t_R.

The number of theoretical plates can be expressed per metre (N'):

$$N' = \frac{N}{l}$$

where l = length of column in metres.

Capacity factor (mass distribution ratio, D_m)
The capacity factor or mass distribution ratio is defined as follows:

$$D_m = \frac{\text{amount of solute in stationary phase}}{\text{amount of solute in mobile phase}}$$

This factor determines the retention of a solute and can be calculated from the chromatogram using the following formula:

$$D_m = \frac{(t_R - t_M)}{t_M}$$

where t_R = retention time of the solute
t_M = retention time of an unretained component

A low D_m value indicates that the peak elutes close to the solvent front, which may compromise selectivity. A minimum D_m value of 1 is recommended for the peak of interest.

The retention time of the test substance can be varied, if necessary, by changing the relative proportion or composition of solvents in the mobile phase. Generally, an increase in the proportion of a more polar solvent will lead to a shorter retention time on a normal-phase column and a longer retention time on a reversed-phase column.

Resolution factor (R_s)
The resolution between two peaks of similar height in a chromatogram can be calculated using the following formula:

$$R_S = \frac{1.18(t_{R2} - t_{R1})}{(W_{b1} + W_{b2})}$$

where t_{R1} and t_{R2} = retention times or baseline distances between the point of injection and the perpendicular dropped from the maximum of each of the two peaks.
W_{b1} and W_{b2} = the respective peak widths determined at half peak height, measured in the same units as t_{R1} and t_{R2}.

The value of R_s for a baseline separation between peaks of similar height should be at least 1.5.

Relative retention
The relative retention (r) is calculated as an estimate using the following formula:

$$r = \frac{t_{R2} - t_M}{t_{R1} - t_M}$$

where t_{R2} = retention time of the peak of interest
t_{R1} = retention time of the reference peak
t_M = retention time of an unretained component

The unadjusted relative retention (r_G) is calculate from the expression:

$r_G = t_{R2}/t_{R1}$

Unless otherwise indicated, values for relative retention stated in the monographs correspond to unadjusted relative retention.

Symmetry factor (A_s)
The symmetry factor for a peak can be calculated using the following formula:

$$A_s = \frac{W_x}{2d}$$

where W_x = peak width at 5% of peak height, measured from the baseline.
d = baseline distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height, measured in the same units as W_x.

Values of A_s which are greater than 2 may lead to incorrect integration, resulting in erroneous quantitation. The main factors that influence peak symmetry depend upon retention, solvent effects, incompatibility of the solute with the mobile phase, or development of an excessive void at the inlet of the column.

In reversed-phase chromatography, adsorption phenomena due to the presence of residual silanol groups in the stationary phase may lead to tailing (poor peak symmetry).

Repeatability
Unless otherwise stated in the "Assay" of the individual monograph, the relative standard deviation of peak areas or peak heights for a series of injections of reference solutions bracketing groups of test solutions should not exceed 2.0%.

In a "Related substances" test the relative standard deviation of peak areas or peak heights for three replicate injections of the reference solution should not exceed 5.0%, unless otherwise stated in the individual monograph.

In a series of injections the relative standard deviation of the retention time of the principal peak should not exceed 1.0%.

Adjustment of chromatographic conditions
The extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods are described below for information. The chromatographic conditions described have been validated during the elaboration of the monograph. The system suitability tests are included to ensure the separation required for satisfactory performance of the test or assay. Nonetheless, since the stationary phases are described in a general way and there is such a variety available commercially, with differences in chromatographic behaviour, some adjustments of the chromatographic conditions may be necessary to achieve the prescribed system suitability requirements and to achieve the indicated retention time. The retention time must be within ±10.0 per cent of that indicated in the test and/or assay. With reverse-phase liquid chromatographic methods, in particular, adjustment of the various parameters will not always result in satisfactory chromatography. In that case, it may be necessary to replace the column with another of the same general type (e.g. octadecylsilyl silica gel) that exhibits the desired chromatographic behaviour.

For critical parameters the adjustments are defined clearly in the monograph to ensure the system suitability.

Multiple adjustments which may have a cumulative effect on the performance of the system are to be avoided.

Composition of the mobile phase The amount of the minor solvent component may be adjusted by ±30% relative or ±2% absolute, whichever is the larger. [*Examples*: For a minor component that is at 10% of the mobile phase, a 30% relative adjustment allows a range of 7–13%, whereas a 2% absolute adjustment allows a range of 8–12%; in this case, the relative value is the larger. For a minor component that is at 5% of the mobile phase, a 30% relative adjustment allows a range of 3.5–6.5%, whereas a 2% absolute adjustment allows a range of 3–7%; in this case, the

absolute value is the larger.] No other component is altered by more than 10% absolute.

pH of the aqueous component of the mobile phase ±0.2 pH, unless otherwise stated in the monograph, or ±1.0 pH when neutral substances are to be examined.

Concentration of salts In the buffer component of a mobile phase: ±10%.

Detector wavelength No adjustment permitted.

Stationary phase

column length: ±70%,

column internal diameter: ±25%,

particle size: maximal reduction of 50%, no increase permitted.

Flow rate ±50%. When the retention time of the principal peak is indicated in a monograph, the flow rate has to be adjusted, if the column internal diameter has been changed. No decrease in flow rate is permitted, however, if the monograph uses apparent number of theoretical plates in the qualification section.

Temperature ±10%, to a maximum of 60 °C.

Injection volume may be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.

Gradient elution The configuration of the equipment employed may significantly alter the resolution, retention time and relative retentions described in the method. Should this occur, it may be due to excessive dead volume (dwell volume) which is the volume between the point at which the two eluants meet and the top of the column.

Recommended procedure

To equilibrate the column, allow the mobile phase to flow through the chromatographic system until the baseline is stable at the flow rate specified in the individual monograph (about 30 minutes). Prepare the prescribed test and reference solutions as directed. Inject the prescribed reference solution and, if necessary, adjust the detector and/or recorder response to produce an adequate peak size. For chart recorders and integrators this should be at least 50% of the full-scale deflection of the principal peak in the chromatograms obtained with the reference solution. Ensure that the criteria for system suitability are met.

The reference solution should be injected at the start, at regular intervals during, and at the end of a series of assays (e.g. every 2–4 samples). Both the reference and the test solutions should be injected in duplicate.

In determining the component composition of a complex mixture, a "normalization" procedure, based on the calculation of individual peak areas as a percentage of the total area of all the peaks, may be used where the relative response factors of the individual components are similar. The response factor is relative, being the response of the equal mass of one substance relative to that of another according to the conditions described in the test. For example, when an HPLC test with UV/vis detection is used for the control of impurities, the wavelength of detection should be such that the substance and its impurities have similar responses. If an impurity has a significantly different response

(more than ±20%) from that of the substance being examined, the preferred manner of limiting this impurity is to use a reference substance of the impurity. If a reference substance is not available, the response factors of the potential impurities relative to those of the substance being examined are determined during method development. Subsequently, the derived correction factors (i.e. the reciprocals of the response factors) are applied, if necessary, as described in the individual monograph.

If gradient elution is specified in the monograph, an instrument equipped with a special pumping system capable of delivering a mobile phase of continuously varied composition is needed. The mobile phase composition changes from the initial composition within a fixed period of time, as specified in the monograph. Where the mobile phase composition is varied at a linear gradient elution, perform a blank run to identify any interfering peaks by injecting the solvent specified for preparing the test solutions. Allow sufficient time for equilibration when the mobile phase is reset to the initial composition for the next injection.

1.14.5 Gas chromatography

Gas chromatography may be regarded as a form of column chromatography in which the mobile phase is a gas (referred to as the carrier gas) rather than a liquid solvent. The stationary phase may be an active adsorbent such as alumina, silica gel, or carbon (gas-solid chromatography) or it may be a liquid that is coated as a thin film on a finely divided inert solid support such as diatomaceous earth, firebrick, glass beads, or other suitable material (gas-liquid chromatography); if the chromatographic column is of very small diameter the stationary phase may be coated on its inner wall to provide the so-called "open tube" or "capillary" column. Certain materials are available that do not require coating with a liquid phase, for example, polyaromatic porous beads, and these are of great value for specific applications.

The substance is introduced in a vaporized state into the carrier gas stream at the head of the column and it undergoes distribution between the gas and liquid or solid stationary phase in a similar manner to that in other forms of chromatography. The partition coefficient (K), which is defined as

$$K = \frac{\text{quantity of solute in the stationary phase}}{\text{quantity of solute in the mobile phase}},$$

will depend upon the nature of the solute, the nature and amount of the stationary phase, the temperature, and the carrier gas flow rate. It will be clear then that the value of K will depend upon the particular column and precise operating conditions used and, since these would be impossible to reproduce exactly from one laboratory to another and from one make of instrument to another, it is necessary to treat the operating conditions set down for pharmacopoeial purposes with a degree of flexibility that is not accorded to other, nonchromatographic, test procedures.

The essential features of the apparatus for effecting gas-liquid chromatography (much more widely used for pharmaceutical applications than adsorption chromatography on a solid) are a source of carrier gas (usually contained in compressed form in a cylinder fitted with a pressure-reducing valve), a flowmeter through which the gas passes, and an injection port that may be heated to a suitable temperature to volatilize but not decompose the substance and through which the test solution is introduced into the flow of carrier gas, preferably directly into the column packing. The constant flow of carrier gas is then maintained as the chromatographic process takes place, the components of the test solution being separated according to the value of K for each under the particular conditions being used.

As the components emerge from the column they enter a suitable differential-type detector, which usually depends on changes of ionization in a flame or on changes of thermal conductivity. Many other types of detectors exist and some of these are useful for specific pharmaceutical applications, for example, the electron capture detector that is of special value in the sensitive detection of halogenated compounds. Electrical signals from the detector are passed to an amplifier connected to a suitable recording device, such as a strip-chart recorder, where signals may be plotted against time. A powerful though very expensive means of detection is to use a mass spectrometer coupled to the gas chromatograph. This is very sensitive and provides information that enables unambiguous identification of substances issuing from the column.

The column is made of glass or metal (in the latter case care has to be taken since certain organic substances undergo metal-catalysed degradation reactions at elevated temperatures) and is contained in a temperature-controlled oven capable of being maintained at temperatures ranging from just above ambient temperature to about 300 °C according to the particular application. Ovens may also be controlled in such a way that a steady rise of temperature may be maintained over a given period of time; such "temperature programming" is frequently of value when complex mixtures of compounds having widely different volatilization characteristics are being examined. Columns of different lengths are commonly employed, varying from as little as 0.5 m to almost 3 m for packed and from 10 to 100 m for capillary columns; for many pharmaceutical applications a column of about 1.5 m is generally used and it may be from 2 to 5 mm in internal diameter.

The packing contained within columns for gas-liquid chromatography has a profound effect on the quality and effectiveness of separations. The solid support, which may vary in particle size from about 75 µm to 250 µm (60–200 mesh; although in any given column it should be closely defined within a narrow range), must be as inert as possible, particularly when polar drugs are to be chromatographed on supports coated with low concentrations of a liquid of low polarity. Active sites on the solid support may result in peak tailing of the solute, or even in its decomposition or rearrangement. Reactivity of the support may be minimized by treatment with a silanizing reagent before it is coated with the stationary phase. Injection residues might render the inlet part of the column ineffective.

Liquid phases commonly used include macrogols (polyethylene glycols) and esters, high molecular weight amides, silicone gums and fluids, and hydrocarbons. The silicone gums are substituted polysiloxanes and are a particularly valuable series of stationary phases. The highest temperature at which the stationary phase is designed to be used should be carefully noted and respected since "column bleeding" may occur and vitiate results if excessive temperatures are used. Prior to use a new column should be conditioned by maintaining it for several hours with a stream of carrier gas passing through it at a temperature somewhat higher than the temperature at which the column is subsequently to be used but certainly not above the highest recommended temperature.

As carrier gas it is necessary to choose one that is inert and for flame-ionization detection (the most commonly used method in pharmaceutical analysis), nitrogen is very satisfactory. Though helium is also suitable and is actually to be preferred for work with a thermal conductivity detector because of its high thermal conductivity, it is expensive and not readily available in all parts of the world, whereas nitrogen is.

For quantitative applications of gas-liquid chromatography in the pharmacopoeia an internal standard is usually used since the comparison of one chromatogram with another resulting from a second injection on to the column could be subject to error. The addition of a suitable internal standard to the test solution and to a standard solution eliminates this error since the ratio of area or height (see below) of the peak due to the substance to be determined and of that due to the internal standard is compared from one chromatogram to another. In other applications it may be more convenient to use a process of normalization (particularly where an impurity is being assessed). In this case, the area of the peak due to the sought-for impurity is expressed as a percentage of the total area of all peaks derived from the substance being examined and its attendant impurities. Since the peak due to the principal component will usually be at least two orders of magnitude greater than that due to a minor impurity, it is necessary for such determinations to use a reliable automatic integrator and a wide-range amplifier that will respond in a linear fashion to both the major and the minor components. Peak areas may also be determined by planimeter, graphically, or by comparing the weights of paper cut from underneath the various peaks. In certain circumstances, it may be valid to use measurements of peak height rather than peak area, although the latter is considered to be more accurate for quantitative determinations. When the measurement of peak width is required, it is defined as the distance along the base line that is between the two points of intersection of lines drawn tangentially to the sides of the peak.

When the internal standard method is used the detailed procedure given below is applicable. It will be noted that reference is made to 3 solutions. The first of these (solution 1) contains an internal standard and an appropriate quantity of the substance to be determined (in the case of an impurity determination this might be the impurity itself, if available, or a suitably low loading of the substance in which impurities are to be sought). Chromatogram A thus

obtained permits the relationship of response to the internal standard and to the substance being determined to be established. The second solution (solution 2) consists only of the substance being examined; this enables the analyst to confirm that there is no impurity present that will elute with the same retention time as the internal standard or to make allowance for the quantity present if there is a coincident peak. The third solution (solution 3) consists of the substance being examined and the internal standard (the latter present at the same concentration as in solution 1). Data derived from chromatograms A and C, corrected if necessary for observations made on chromatogram B, enable the quantities of minor constituents present in the substance being examined to be determined.

When a normalization procedure is used, a single solution may suffice since the total area of all minor peaks is to be expressed as a proportion of the total peak area. If a specific minor peak is to be evaluated, a second solution containing the sought-after material will be necessary so that the appropriate peak on the chromatogram of the substance being examined may be identified.

Recommended procedure

The length of the chromatographic column, the stationary phase, the solid support, the temperature, the carrier gas, the detector, and any other relevant details that are required for the determination are specified in the monograph. Where a non-volatile material is to be injected on to the column, a suitable interchangeable pre-column may be used.

In certain monographs a minimum column efficiency may be required. This is defined by the expression $16\ t_R^2/Ly^2$, where

t_R is the distance (in mm) along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak produced by the internal standard specified in the monograph;
L is the length (in m) of the column;
y is the peak width (in mm) of the peak produced by the internal standard.

When aqueous solutions are examined and a flame ionization detector is used, the results are invalid if the water is eluted at the same time as any of the required components.

Method

Using solution 1, determine experimentally suitable sensitivity settings of the instrument and the volumes of solutions to be injected to produce an adequate response. Adjustment of the concentration of the internal standard should be made, if necessary, so that the recorder response produced by the internal standard is approximately the same as the response of the substance being determined. Prepare a differential chromatogram by injecting the selected volume of solution 1 through the sample injection port on the chromatographic column,

maintained at a suitable temperature, and eluting with the carrier gas. Repeat the determination twice more. In the same manner, prepare graphs using the same volumes of solutions 2 and 3. Measure the peak areas or, where the symmetry factor lies between 0.95 and 1.05, the peak heights produced by the substance or substances being determined and the internal standard. If an impurity peak has the same retention time as the internal standard, allowance is made in assessing the responses of these constituents for the contribution of each. From the values obtained calculate the proportion of the substance or substances being determined.

The symmetry factor of a peak is calculated from the expression $y_x/2A$, where:

y_x is the width of the peak at one-twentieth of the peak height;
A is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

The results of the determination are usually not valid unless the resolution between measured peaks on the chromatogram is greater than 1.0. The resolution is calculated from the expression $2(t_{Rb} - t_{Ra})/(Y_a + Y_b)$, where:

t_{Ra} and t_{Rb} are the distances along the baseline between the point of injection and perpendiculars dropped from the maxima of two adjacent peaks;
Y_a and Y_b are the respective peak widths.

Static Head-space Gas Chromatography

Static head-space gas chromatography is a technique particularly suitable for separating and determining volatile compounds present in solid or liquid samples. The method is based on the analysis of the vapour phase in equilibrium with the solid or liquid phase.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

Apparatus

The apparatus consists of a gas chromatograph provided with a device for introducing the sample that may be connected to a module that automatically controls the pressure and the temperature. If necessary, a device for eliminating solvents can be added.

The sample to be analysed is introduced into a container fitted with a suitable stopper and a valve-system which permits the passage of the carrier gas. The container is placed in a thermostatically controlled chamber at a temperature set according to the substance to be examined.

The sample is held at this temperature long enough to allow equilibrium to be established between the solid or liquid phase and the vapour phase.

The carrier gas is introduced into the container and, after the prescribed time, a suitable valve is opened so that the gas expands towards the chromatographic column taking the volatilized compounds with it.

Instead of using a chromatograph specifically equipped for the introduction of samples, it is also possible to use airtight syringes and a conventional chromatograph. Equilibration is then carried out in a separate chamber and the vapour phase is carried onto the column, taking the precautions necessary to avoid any changes in the equilibrium.

Recommended procedure

Using the reference preparations, determine suitable instrument settings to produce an adequate response.

Method 1: Direct calibration

Separately introduce into identical containers the preparation to be examined and each of the reference preparations, as prescribed in the monograph, avoiding contact between the sampling device and the samples.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

Method 2: Standard additions

Add to a set of identical suitable containers equal volumes of the preparation to be examined. Add to all but one of the containers suitable quantities of a reference preparation containing a known concentration of the substance to be determined so as to produce a series of preparations containing steadily increasing concentrations of the substance.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the substance to be determined in the preparation to be examined.

Alternatively, plot on a graph the mean of readings against the added quantity of the substance to be determined. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of the substance to be determined in the preparation to be examined.

1.15 Electrophoresis

Electrophoresis is a physical method of analysis permitting the separation of compounds that are capable of acquiring en electrical charge in a conducting electrolyte. In this medium the ionized particles move more or less rapidly under the influence of an electrical field.

The electrophoretic mobility is the rate of migration of the substance measured in cm/s under the influence of a potential gradient of 1 V/cm, and is expressed in $cm^2 \cdot V^{-1} \cdot s^{-1}$.

The measurement of electrophoretic mobility is significant only where experimental conditions have been precisely defined. This mobility depends on the characteristics of the substance, its nature, size, form, and electrical charge. It also depends on the composition of the conducting liquid, its nature, concentration, pH, the presence of additional solvents and viscosity. The direction of migration depends on the sign of the electrical charge of the particle as it moves towards the electrode of opposite sign.

According to the methods used, the electrophoretic mobility is either measured directly or compared with that of a reference substance.

Moving boundary (free-flow) electrophoresis

This technique, used exclusively for the determination of the mobility, is particularly suitable for substances of high molecular weight with poor diffusion properties.

The boundaries are usually measured both before and after the application of an electrical field by a physical method, such as refractometry or conductometry. The concentration of the substance in the conducting liquid, the characteristics of the latter and the details of the procedure, including quantitative evaluation of the fractions, are specified in the monographs.

Zone electrophoresis (electrophoresis using a supporting medium)

This method uses only small sample sizes. The nature of the supporting medium (for example, paper, cellulose acetate, starch-gel, agar-gel, polymethacrylamide, mixed gel) introduces additional factors influencing the mobility. The rate of migration depends on the mobility of the particles and also on the electroendosmotic current (in the case of carriers with polar properties), on the currents due to evaporation (caused by heat generated through the Joule effect), and on the gradient of the electrical field.

In practice, the mobility of the electrophoretic zones and their signs are ignored; the zones are located by experience or by comparison with those given by a reference substance treated in the same way.

After separation of the constituents the position of colourless substances may be determined by treating the electropherogram with a reagent that will convert them to coloured or fluorescent derivatives. For quantitative purposes,

the spot (zone) may be carefully separated, the substance eluted with a suitable solvent and then determined by a sufficiently sensitive method, such as spectrophotometric measurement, either directly or after a chemical reaction. In another quantitative procedure after conversion to a coloured derivative, the zone intensity can be measured with the aid of a scanning densitometer.

An apparatus for electrophoresis on a supporting medium is composed of:

- A source of direct current, preferably of stabilized voltage.
- A chamber for electrophoresis, generally in the form of a parallelepiped, made from glass or rigid plastic material with an airtight lid ensuring the maintenance of an atmosphere of saturated humidity. Two insulated electrical leads are sealed through the walls of the chamber, one at either end, each lead having an internal connector to which are attached electrodes of platinum wire. The chamber should be fitted with suitable safety devices to ensure that the electrical supply is disconnected when the lid is removed. Two double troughs provided with a central lengthwise partition are inserted in the chamber, one at each end. Alternatively, the troughs may be integral parts of the chamber. One platinum electrode is laid along the bottom of each outer trough compartment. The electrodes are connected through external insulated cables to an electrical power source having an output of not less than 450 V D.C. at 150 mA. The power source should be provided with a means of indicating and controlling the voltage and of indicating the current consumption. Additional circuitry may be incorporated to stabilize the voltage.
- A holder device. When paper or cellulose acetate is used, the carrier strips impregnated with the conducting liquid are stretched by an appropriate arrangement and the ends immersed into the electrode troughs. In gel electrophoresis, an adherent on an even layer of gel is placed on glass and the electrical connexions are attached at each end.
- A device to locate and measure the spots.

Recommended procedures

Paper electrophoresis

A chamber about 50 cm long, 38 cm wide and 4.5 cm deep, with troughs about 37 cm long externally, 5 cm wide and 2 cm in depth internally, is suitable.

The electrophoresis paper consists of suitable filter-paper (Whatman 3 MM or similar grade is suitable) that has been washed chromatographically with a suitable solvent if so specified in the monograph. The paper is cut into strips of appropriate size and a baseline is drawn across the paper about 13 cm from one end.

Fill the troughs of the apparatus with the conducting liquid specified in the monograph. Place strips of electrophoresis paper (about 30 cm by 5 cm) in the troughs so as to form bridges between the outer and inner compartments and ensure that the electrodes are fully immersed in the conducting liquid in the outer compartments.

Apply separately to points along the base line of the electrophoresis paper, at least 1 cm from the edge of the paper and not less than 2.5 cm apart, the volumes of solutions prepared as specified in the monograph.

Allow the spots to dry and then place the end of the paper nearest the baseline in the inner compartment of the trough connected to the anode and the other end in the inner compartment of the trough connected to the cathode. Wet the paper with the conducting liquid, using a brush, starting from the ends of the paper and working towards the baseline. Do not wet the strip that includes the applied substance. Close the lid, allow the liquid to diffuse across the baseline, if necessary cover the apparatus so as to exclude light, connect the cables to the power supply and switch on the current. Adjust the voltage to about 20 V per cm of paper between the troughs and allow electrophoresis to proceed for the time indicated or until the marker substances have moved the specified distances. Switch off the current, remove the paper, dry in a current of air protected from light if necessary, and examine the resulting electropherogram under the conditions prescribed in the monograph. When the use of marker substances is specified, the test is valid only if the marker substances move to the specified distances from the baseline. If the intensity of any subsidiary spots derived from the tested substance is less than that of the spot obtained from the reference solution, the substance conforms to the requirements. When specified in the monograph, spray the paper uniformly on both sides with the reagent, carry out any further prescribed treatment to complete the reaction, and apply the same criteria to the resulting spots.

Electrophoresis on cellulose acetate strips

It is preferable to use a smaller chamber than for paper electrophoresis; one measuring about 25 cm by 24 cm with troughs of 10 cm by 23 cm is suitable.

Use cellulose polyacetate strips of suitable quality, measuring 2.5 cm by 17 cm, which are immersed in the conducting liquid for approximately 1 hour before use.

Apply the solutions, prepared as specified in the monograph and in the volume indicated, 8 cm from one end of the strip, then carry out electrophoresis as described under paper electrophoresis. Colour the bands, wash them and render them transparent by the methods specified in the monographs, which also give the method of evaluation to be used.

Gel electrophoresis

The inert carrier consists of a 1–2 mm thick layer of an agar or starch gel of suitable consistency and shaped as an elongated rectangle.

The conducting liquid is either incorporated into the gel layer or sprinkled on it, until it is well moistened, after it has already been formed. The solution of the substance is placed on the surface of the gel layer or inside the holes bored for that purpose in the layer. The gel layer is connected at both its narrower ends with two troughs containing the conducting liquid, the connection being made by wicks composed of a double layer of absorbent lint moistened

with the conducting liquid. The gel layer on its support and the connections are then placed in a suitable chamber.

The electrophoretic process is effected by application of direct electric current. To remove heat generated by the Joule effect of the current, water or other suitable cooling liquid should be circulated in the course of the process through the supporting plate.

When the process has been completed, the resulting spots or areas of migration are located by a suitable method (for example, as an inhibition zone after suitable incubation, if the tested substance is an antibiotic and an appropriate test organism has been incorporated into the gel layer, or by a chemical method), as specified in the individual monographs.

1.16 Phase solubility analysis

Phase solubility analysis is a technique for quantitatively determining the purity of a substance through the application of precise solubility measurements. At a given temperature, a definite amount of a pure substance is soluble in a definite quantity of solvent. The resulting solution is saturated in respect of the particular substance, but the solution remains unsaturated in respect of other substances even though such substances may be closely related in chemical structure and physical properties to the particular substance being tested. Constancy of solubility indicates that a material is pure or free from foreign substances except in the unique case where the percentage composition of the material being tested is in direct ratio to the solubilities of the respective components. Conversely, variability of solubility indicates the presence of an impurity or impurities.

The standard solubility method consists of several distinct steps: (*a*) preparation of a series of separate systems composed of increasing quantities of material and measured, constant amounts of solvent; (*b*) establishment of equilibrium for each system at identical constant temperature and pressure; (*c*) separation of the solid phase from the solutions; (*d*) determination of the concentration of the material dissolved in the various solutions; (*e*) graphical representation of the ratio of the weight of the dissolved materials per weight of solvent against the ratio of the total weight of material per weight of solvent, extrapolation and calculation. Alternatively, statistical procedures may be used to evaluate the purity of the test substance from the data obtained.

Solvents

The following criteria are used in the selection of a proper solvent for phase solubility analysis:

(1) The solvent should be of such volatility that it can be evaporated under vacuum, but it should not be so volatile that difficulty is experienced in transferring and weighing the solvent and its solutions. Normally, solvents with boiling points between 60 °C and 150 °C are suitable.

(2) The solvent should not adversely affect the substance. Solvents that cause decomposition or react with the substance cannot be used. Solvents that solvate or form salts should be avoided if possible.
(3) The solvent should be of known purity and composition. Mixed solvents are permissible. Trace impurities may affect solubility greatly.
(4) For the method described below the test substance should be soluble to the extent of not less than 4 mg/g and not more than 50 mg/g in the solvent chosen. A solubility of 10–20 mg/g is optimal.

Apparatus

Constant-temperature bath. For these tests use a constant-temperature bath capable of maintaining a preselected temperature within ±0.1 °C. Normally temperatures of 25 °C or 30 °C are selected. The bath should be equipped with a horizontal shaft capable of rotating at approximately 25 revolutions per minute, equipped with clamps to hold ampoules. Alternatively the bath may contain a suitable vibrator, capable of vibrating at 100–120 vibrations per second, equipped with a shaft with suitable clamps to hold the ampoules, or other suitable device to achieve equilibrium in the ampoules.

Ampoules. Use 15-ml ampoules similar to that shown in Fig. 2. Other containers may be used provided that they are leakproof and are suitable in all other respects.

Solubility flasks. Use solubility flasks suitable for freeze-drying. A suitable flask (with stopper) is shown in Fig. 2.

Balance. Use a suitable balance and technique to ensure that all weighings are within ±10 µg.

Recommended procedure

The method described below is generally applicable. However, in certain cases other conditions (volume of solvent, etc.) than those specified here might be preferable.

System composition

Weigh accurately not less than 7 marked, scrupulously cleaned, ampoules and into each of them weigh, accurately, increasingly larger amounts of the test substance. The weight of the test substance is selected so that the first ampoule contains slightly less material than will go into solution in 5 ml of the selected solvent, and the second ampoule and subsequent ampoules slightly more than the indicated solubility. Pipette 5.0 ml of the solvent into each of the ampoules, cool in a dry-ice/acetone mixture, and seal, using a double-jet air-gas burner and taking care to save all the fragments of glass. Allow the ampoules and their contents to come to room temperature, and weigh the individual sealed ampoules plus any corresponding glass fragments. Calculate the system composition, in mg of substance per g of solvent, for each ampoule by the formula $1000\ (W_2 - W_1)/(W_3 - W_2)$, in which W_1 is the weight of the empty ampoule,

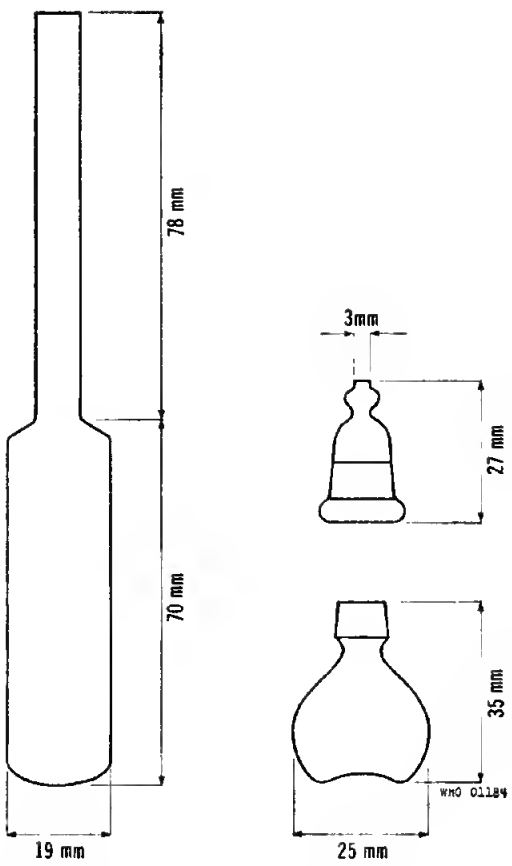


Figure 2. Ampoule (left) and solubility flask (right) used in phase solubility analysis.

W_2 is the weight of the ampoule plus test substance, and W_3 is the weight of the ampoule plus test substance, solvent and separated glass.

Equilibration

The time required for equilibration varies with the substance, the method of mixing (vibration or rotation) and the temperature. Normally equilibrium is obtained more rapidly by the vibration method (1–7 days) than by the rotational method (7–14 days).

In order to demonstrate that a state of equilibrium has been attained, the following procedure is frequently applicable: Bring about a supersaturated solution in one of the ampoules – the next to the last in the series – by warming it to a temperature about 10 °C above that of the constant-temperature bath, taking care that not all the solid in the ampoule is dissolved. Thereafter, treat this ampoule in exactly the same manner as the other ampoules. If the solubility value obtained from it plots out in line with the other values, it indicates that all the ampoules have attained equilibrium. However, failure of the solubility value from the "supersaturated" ampoule to fall in line with the other solubility values does not necessarily prove that the other ampoules have not

attained equilibrium, since it can occasionally be due to the tendency of certain materials to remain in supersaturated solution. To attain equilibrium in such cases, run a series of phase solubility analyses applying different equilibration times so as to make certain that constant values for the slope of the solubility curve have been achieved.

Solution composition

After equilibration, place the ampoules vertically in a rack in the constant-temperature bath, with their necks above the water level, and allow the contents to settle. Taking full precautions to minimize solvent evaporation, open the ampoules, and remove a volume of 2.0 ml from each by means of a pipette equipped with a small pledget of cotton-wool or with other suitable means of serving as a filter. Remove the cotton-wool, transfer the aliquot of clear solution from each ampoule to a marked, tared solubility flask, and weigh each flask plus its solution to obtain the weight of the solution. Cool the flasks in a dry-ice/acetone bath, and then evaporate the solvent in a vacuum. Gradually increase the temperature from 70 to 100 °C, and dry the residue to constant weight. Calculate the solution composition, in mg of substance per g of solvent, by the formula $1000\ (F_3 - F_1)/(F_2 - F_3)$, in which F_1 is the weight of the solubility flask, F_2 is the weight of the flask plus solution, and F_3 is the weight of the flask plus residue.

Calculation

Prepare a graphical representation of the results for each portion of the test substance taken by plotting the ratio of the weight of the dissolved materials per weight of solvent (Y axis or solution composition) against the ratio of the total weight of material per weight of solvent (X axis or system composition). As shown in Fig. 3, the points for those containers that represent a true solution should approach a straight line (AB) with a slope of 1, passing through the origin; the points corresponding to saturated solutions should approach another straight line (BC), the slope, S, of which represents the fraction of total impurities present in the test substance. Failure of points to approach a straight line usually indicates that equilibrium has not been achieved although this may also be due to formation of a solid solution or departure from ideal behaviour. Calculate the percentage purity of the test substance by the formula $100 - 100S$. The slope may be calculated by the equation $S = (Y_2 - Y_1)/(X_2 - X_1)$ in which Y_2 and Y_1 represent solution compositions, and X_2 and X_1 represent the respective system compositions, at convenient points on the second straight line (BC).

The point B in the diagram represents the system where the main component or, in rare cases, the least soluble of the components of the test substance has reached the limit of its solubility. The solubility of this component is obtained by extending the solubility line (BC) through the Y axis. The point of interception on the Y axis gives the solubility, in mg/g, which should be constant for a given compound.

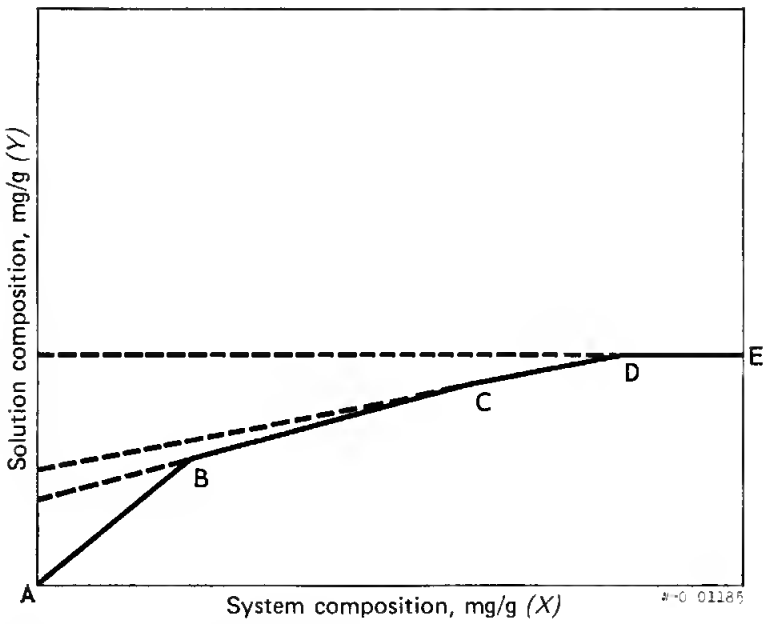


Figure 3. Typical phase solubility diagram.

At point C in the diagram the next component of the test substance has reached the limit of its solubility. The intercept obtained by extending the solubility line (CD) through the *Y* axis represents the combined solubilities of the two components that have first reached their solubility limits. The solubility of the second component can thus be obtained by subtraction.

Between points D and E in the diagram the solution is saturated in respect of all components in the test substance, and its composition remains constant.

Under appropriate conditions the number of impurities present in the test substance is shown by the number of inflexions of the solubility curve above the first saturation point (B) and the solubilities of the respective components can be obtained in a way similar to that described above.

2. Chemical methods

2.1 General identification tests

Acetylated substances

Place a quantity of the substance as specified in the monograph in a test-tube (of maximum 18 mm diameter) and treat it with 3 drops of phosphoric acid (~1440 g/l) TS. Close the tube with a stopper through which passes a smaller test-tube filled with water and on the outside of which hangs a drop of lanthanum nitrate (30 g/l) TS. Heat the apparatus in a boiling water-bath for 5 minutes. Transfer the drop of lanthanum nitrate to a white porcelain spot plate and mix with a drop of iodine (0.02 mol/l) VS. Place at the edge of the mixture

a drop of ammonia (~100 g/l) TS. A blue colour slowly appears at the interface of the two liquids and persists for a short time.

Amines, primary aromatic
Dissolve a quantity of the substance as specified in the monograph in 2 ml of hydrochloric acid (~70 g/l) TS with the aid of heat, if necessary. Cool in ice, treat it with 4 ml of sodium nitrite (10 g/l) TS and pour the mixture into 2 ml of 2-naphthol TS1 containing 1 g of sodium acetate R. A heavy precipitate, coloured as specified in the monograph, is produced.

Ammonia and volatile aliphatic amines
Dissolve a quantity of the substance as specified in the monograph, place the solution in a test-tube and add 1 g of magnesium oxide R; warm, if specified in the monograph. Alkaline vapours evolve gradually and turn manganese/silver paper R black, the reagent paper being placed in the upper part of the test-tube.

Ammonium
Carry out the test in an apparatus consisting of stoppered test-tubes A and B connected by a bent glass tube to permit a stream of air to pass consecutively through test-tubes A and B.

Place the solution as specified in the monograph and 0.2 g of magnesium oxide R into test-tube A, and 1 ml of hydrochloric acid (0.1 mol/l) VS containing 1 drop of methyl red/ethanol TS in test-tube B. Bubble air through the apparatus. Evolved ammonia turns the colour of the solution in test-tube B to yellow. On the addition of 1 ml of sodium cobaltinitrite (100 g/l) TS to this solution a yellowish brown precipitate is formed.

Bismuth

A. Prepare the solution in hydrochloric acid (~250 g/l) TS as specified in the monograph and dilute 10 times with water. A white precipitate is formed, which turns dark brown on the addition of sodium sulfide TS.
B. Treat the solution in nitric acid (~1000 g/l) TS as specified in the monograph with potassium iodide (80 g/l) TS. A black precipitate is formed, which is soluble in an excess of the reagent to give a yellowish brown or orange solution. Dilute this solution with several volumes of water and heat; an orange or copper-coloured precipitate is obtained. The black precipitate that is first formed on the addition of potassium iodide (80 g/l) TS also becomes orange or copper-coloured when heated with water.

Bromides

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/l) TS and add silver nitrate (40 g/l) TS. A yellowish curdy precipi-

tate is produced, which is partially soluble in ammonia (~260 g/l) TS, but almost insoluble in ammonia (~100 g/l) TS and in nitric acid (~1000 g/l) TS.

B. (For testing bromides or hydrobromides of insoluble or sparingly soluble bases.) Prepare the solution as specified in the monograph, add ammonia (~100 g/l) TS, filter, acidify the filtrate with nitric acid (~130 g/l) TS, and proceed with test A.

C. Prepare the solution as specified in the monograph, acidify with sulfuric acid (~100 g/l) TS, and mix with chlorine TS. A brown solution results; after shaking with chloroform R it becomes colourless, whereas the chloroform layer turns reddish.

Calcium

A. Prepare the solution as specified in the monograph and add to it ammonium oxalate (25 g/l) TS. A white precipitate is formed, which is soluble in hydrochloric acid (~250 g/l) TS but is practically insoluble in acetic acid (~300 g/l) TS.

B. Treat 1 drop of a solution as specified in the monograph with 4 drops of glyoxal bis(2-hydroxyanil) TS, and 1 drop of sodium hydroxide (~80 g/l) TS. A reddish brown precipitate is formed, which dissolves in chloroform R to give a red solution.

Chlorides

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/l) TS and add silver nitrate (40 g/l) TS. A white curdy precipitate is produced, which is soluble in ammonia (~100 g/l) TS but is practically insoluble in nitric acid (~1000 g/l) TS.

B. (For testing chlorides or hydrochlorides of insoluble or sparingly soluble bases.) Prepare the solution as specified in the monograph, add ammonia (~100 g/l) TS, filter and acidify the filtrate with nitric acid (~130 g/l) TS and proceed with test A.

C. Mix the quantity of the substance as specified in the monograph with an equal quantity of manganese dioxide R, moisten with sulfuric acid (~1760 g/l) TS and heat gently. The evolved chlorine is recognizable by its greenish colour and produces a blue coloration of moistened starch/iodide paper R. Carry out the reaction preferably under a hood.

Citrates

A. Treat at ambient temperature a neutral solution as specified in the monograph with calcium chloride (55 g/l) TS. No precipitate is formed, but on boiling, a white solid is produced, which is soluble in acetic acid (~300 g/l) TS.

B. Boil a solution with mercuric sulfate TS as specified in the monograph and filter if necessary. After the addition of a few drops of potassium permanganate (10 g/l) TS to the filtrate, the colour is discharged and a white precipitate is produced.

Ferrous salts

A. Prepare a solution as specified in the monograph and add potassium ferricyanide (10 g/l) TS. A dark-blue precipitate is formed, which is practically insoluble in hydrochloric acid (~70 g/l) TS.
B. Prepare a solution as specified in the monograph, acidify with sulfuric acid (~100 g/l) TS, and treat with *o*-phenanthroline (1 g/l) TS. An intense red colour is produced, which is discharged by the addition of ceric sulfate (35 g/l) TS.

Iodides

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/l) TS and add silver nitrate (40 g/l) TS. A yellow curdy precipitate is formed, which is practically insoluble in ammonia (~100 g/l) TS and in nitric acid (~1000 g/l) TS.
B. (For testing iodides of insoluble or sparingly soluble bases.) Prepare the solution as specified in the monograph, add ammonia (~100 g/l) TS, filter and acidify the filtrate with nitric acid (~130 g/l) TS and proceed with test A.
C. Prepare a solution as specified in the monograph, acidify with sulfuric acid (~100 g/l) TS and add potassium nitrite (100 g/l) TS. A brown solution results; after shaking with chloroform R, it becomes colourless, whereas the chloroform layer turns violet.

Nitrates

A. Prepare a solution as specified in the monograph and treat it with ferrous sulfate (15 g/l) TS. No brown colour appears unless sulfuric acid (~1760 g/l) TS is cautiously added to form a lower layer. A brown colour is then produced at the interface of the two liquids.
B. Add 2 mg of the finely ground test substance to a mixture of 0.1 ml of nitrobenzene R and 0.2 ml of sulfuric acid (~1760 g/l) TS. Allow to stand at room temperature for 5 minutes, cool in ice, and add slowly while mixing 5 ml of water and 3 ml of sodium hydroxide (~400 g/l) TS. Add 5 ml of acetone R, shake and allow to separate. An intense violet colour is produced in the upper phase.

Orthophosphates

A. Add drop by drop a quantity of nitric acid (~130 g/l) TS to 5 ml of ammonium molybdate (95 g/l) TS until any precipitate that may appear dissolves. Divide this solution into 2 portions, add to one portion the test solution acidified with nitric acid (~130 g/l) TS as specified in the monograph, and boil both portions. A yellow precipitate is formed with the test solution while the other shows no more than a slight opalescence.
B. Prepare a neutral solution as specified in the monograph and add silver nitrate (40 g/l) TS. A yellow precipitate is produced, which does not

darken upon heating the solution to boiling. The precipitate is soluble in ammonia (~100 g/l) TS and in nitric acid (~130 g/l) TS.

Potassium
Prepare an alkaline solution as specified in the monograph and treat it with sodium tetraphenylborate (30 g/l) TS. A white precipitate is produced.

Salicylates
Treat a neutral solution as specified in the monograph with ferric chloride (25 g/l) TS. An intense reddish violet colour appears, which remains on the addition of a small amount of acetic acid (~300 g/l) TS but disappears on the addition of hydrochloric acid (~70 g/l) TS, with separation of a white crystalline precipitate.

Sodium
A. Moisten a quantity of the substance with hydrochloric acid (~250 g/l) TS. An intense yellow colour is produced when the solution is introduced into a nonluminous flame.
NOTE: Perform test B if for technical reasons test A cannot be carried out.
B. Acidify a solution as specified in the monograph with acetic acid (~60 g/l) TS, filter, if necessary, and treat it with uranyl/zinc acetate TS. A yellow crystalline precipitate is produced.

Sulfates
A. Prepare a solution as specified in the monograph and add barium chloride (50 g/l) TS. A white precipitate is formed, which is practically insoluble in hydrochloric acid (~250 g/l) TS.
B. To a solution as specified in the monograph, add lead acetate (80 g/l) TS. A white precipitate is formed, which is soluble in ammonium acetate (80 g/l) TS and in sodium hydroxide (~80 g/l) TS, but practically insoluble in hot water.

Tartrates
A. Acidify a solution as specified in the monograph with acetic acid (~300 g/l) TS and add 1 drop of ferrous sulfate (15 g/l) TS, a few drops of hydrogen peroxide (~60 g/l) TS, and enough sodium hydroxide (~80 g/l) TS to make the solution alkaline. A purple or violet colour is produced.
B. Mix a few ml of sulfuric acid (~1760 g/l) TS with a few drops of resorcinol (20 g/l) TS and a few drops of potassium bromide (100 g/l) TS and add 2 or 3 drops of a solution as specified in the monograph. Warm the liquid in a water-bath for 5 to 10 minutes. An intense blue colour is produced. Cool the liquid and pour it into water. The solution becomes red.

2.2 Limit tests

2.2.1 Limit test for chlorides

The limit test for chlorides is provided to demonstrate that the content of chlorides does not exceed the limit given in the individual monograph in terms of micrograms of chloride ions per gram of the substance being tested. The standard solution against which the comparison of opalescence is made contains 250 µg of Cl^-.

Recommended procedure

Carry out the test in matched flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter bearing a 45-ml and a 50-ml mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects.

Prepare a solution as specified in the monograph, transfer to a comparison tube, dilute to 50 ml with water and add 1 ml of silver nitrate (40 g/l) TS. Stir immediately with a glass rod, and set aside for 5 minutes, protected from direct sunlight. The opalescence produced is not greater than the similarly prepared standard opalescence when viewed down the vertical axis of the tube in diffused light against a black background.

Standard opalescence
Measure 5.0 ml of hydrochloric acid CITS and 10 ml of nitric acid (~130 g/l) TS into a comparison tube. Dilute to 50 ml with water, and add 1 ml of silver nitrate (40 g/l) TS. Stir immediately with a glass rod and set aside for 5 minutes, protected from direct sunlight.

2.2.2 Limit test for sulfates

The limit test for sulfates is provided to demonstrate that the content of sulfates does not exceed the limit given in the individual monograph in terms of micrograms of sulfates per gram of the substance being tested.

The solution against which the comparison of turbidity is made contains 480 µg of SO_4^{--} more than the standard barium sulfate suspension.

Recommended procedure

Carry out the test in matched flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter bearing a 45-ml and a 50-ml mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects.

Prepare a solution as specified in the monograph, transfer to a comparison tube, dilute to 45 ml with water and add 5 ml of barium sulfate suspension TS.

Stir immediately with a glass rod, and set aside for 10 minutes. The turbidity produced is not greater than the similarly prepared standard turbidity when viewed down the vertical axis of the tube in diffused light against a black background.

Standard turbidity
Measure 1.00 ml of sulfuric acid (0.005 mol/l) VS and 3 ml of hydrochloric acid (~70 g/l) TS into a comparison tube. Dilute to 45 ml with water, and add 5 ml of barium sulfate suspension TS. Stir immediately with a glass rod and set aside for 10 minutes.

2.2.3 Limit test for heavy metals

The limit test for heavy metals is provided to demonstrate that the content of metallic impurities that are coloured by hydrogen sulfide does not exceed the heavy metals limit given in the individual monograph in terms of micrograms of lead per gram of the test substance.

The test consists of two consecutive operations: preparation of the test solution, and the colour development by reaction with hydrogen sulfide, followed by comparison of the colour obtained with that produced with standard lead solution.

The preparation of the test solution is carried out, as specified in the monograph, according to procedures 1 to 4 described below. A blank is prepared in a similar manner.

The reaction with hydrogen sulfide is carried out by mixing the test solution with freshly prepared hydrogen sulfide TS. The comparison of the colour thus obtained is carried out either by directly comparing the coloration of the liquid in suitable comparison tubes (Method A) or by comparing the intensity of coloration of spots obtained by filtering the liquid using an appropriate apparatus (Method B).

Method A is generally applicable only when the amount of heavy metals in the weight of test substance used exceeds 5 µg; for amounts of 2–5 µg of heavy metals Method B should be used.

The standard lead solution used in the test; dilute lead PbTS contains 10 µg of lead in 1 ml. When 0.1 ml of this solution is employed to prepare the standard for comparison with a solution of 1 g of the substance being tested, the standard solution thus prepared contains 1 µg of Pb and represents the equivalent of 1 µg of lead per g of the substance tested.

Apparatus
For determination of heavy metals by Method A carry out the test in matched flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter bearing a 40-ml and a 50-ml mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in inter-

nal diameter and in all other respects. For mixing the solution use a stirring rod preferably having a loop at the lower end.

For determination of heavy metals by Method B use a 50-ml syringe made of suitable material (usually plastic) with a detachable plunger and a male Luer conical joint of 9 mm internal diameter at the lower end (Millipore syringe XX 11 050 05 is suitable) to which an adaptor for filtration is attached.

The adaptor is made of suitable material (a filtration adapter Millipore SX00 013 00 in polypropylene is suitable) and has a female joint for connecting it with the syringe. It is devised so as to be separable into two parts to permit the exchange of filters, the lower part containing a support for membrane filters 13 mm in diameter. A suitable prefilter (Millipore prefilter AP 2001 300 is suitable) and a membrane filter made of mixed cellulose esters, 13 mm in diameter, with a pore opening of 3 µm (a Millipore filter SSWP 013 00 is suitable) are used for the filtration.

Recommended procedures

Preparation of test solution

Procedure 1. Weigh the quantity of substance specified in the monograph, dissolve it in 25 ml of water, adjust the pH of the solution to 3–4 with acetic acid (~60 g/l) PbTS, or with ammonia (~100 g/l) PbTS, as necessary, then dilute to 40 ml with water and mix.

Procedure 2. Weigh the quantity of substance specified in the monograph, dissolve it in about 30 ml of solvent specified (ethanol (~750 g/l) TS, methanol R, acetone R, or dioxan R may be used), add 0.5 ml of acetic acid (~300 g/l) TS, and dilute to 40 ml with the solvent.

Procedure 3. Place the quantity of the substance specified in the monograph in a suitable crucible, preferably made of silica, and carefully ignite at a low temperature until the contents are thoroughly charred. The crucible may be loosely covered with a lid during the charring. Add to the contents of the crucible 2 ml of nitric acid (~1000 g/l) TS and 5 drops of sulfuric acid (~1760 g/l TS), and cautiously heat until white fumes are evolved, and then ignite, preferably in a muffle furnace, at 500 °C until all the carbon is burned off. Cool, add 2 ml of hydrochloric acid (~250 g/l) TS, and slowly evaporate in a water-bath to dryness. Moisten the residue with 1 drop of hydrochloric acid (~250 g/l) TS, add 10 ml of hot water, and digest for 2 minutes. Add, drop by drop, ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, drop by drop, acetic acid (~60 g/l) PbTS, to adjust the pH to between 3 and 4. Filter if necessary, wash the crucible and the filter with about 10 ml of water, dilute with water to 40 ml, and mix.

Procedure 4. Place the quantity of substance specified in the monograph in a suitable crucible, preferably made of silica, mix it well with about 0.5 g of magnesium oxide R and incinerate until a homogeneous white mass is obtained. If after 15 minutes of incineration the residue is still coloured, let the crucible cool, mix the contents well with a glass rod and resume heating. Next, dissolve the residue in hydrochloric acid (~70 g/l) TS, add, drop by drop, a solution of

ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, also drop by drop, acetic acid (~60 g/l) PbTS, to adjust the pH to 3–4, filter, dilute with water to 40 ml, and mix.

Colour development and measurement

Method A

To 40 ml of the liquid contained in the comparison tube add 10 ml of freshly prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

In another comparison tube place a volume of solution of dilute lead PbTS, containing the lead equivalent of the heavy metals limit specified in the monograph, dilute with water, adjust the pH with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3–4; dilute with water or the solvent used to 40 ml, mix, add 10 ml of freshly prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

Compare the colours by viewing down the vertical axis of the tube in diffused light against a white background, or by another suitable method. The colour of the test solution is not darker than that of the lead standard.

Method B

Take the filtration syringe, arrange the prefilter and the membrane filter as indicated in the diagram for prefiltration, remove the plunger from the syringe, place the test solution inside the syringe, replace the plunger, and filter the test solution slowly by exerting a regular pressure on the plunger. Collect the filtrate in a beaker or a test-tube. Open the adapter and check whether the membrane filter is free from impurities. If not, replace it and repeat the operation in the same manner. Then rearrange the prefilter and membrane filter as indicated in Fig. 4. Adjust the pH of the filtrate with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3–4, add 10 ml of freshly prepared hydrogen sulfide TS, all the reagents previously filtered through a membrane filter, mix, allow to stand for 5 minutes, take out the plunger, place the solution inside the syringe, and filter it through the membrane filter by exerting slowly a regular and moderate pressure on the plunger. Open the adaptor and take out the membrane filter.

Take a volume of solution of dilute lead PbTS containing the lead equivalent to the heavy metals limit specified in the monograph, dilute with water, adjust the pH with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3–4, dilute with water to 40 ml, mix, and proceed as described above.

Compare the intensity of the coloration of spots obtained on the membrane filters. The colour obtained from the test solution is not more intense than that from the lead standard.

2.2.4 Limit test for iron

The limit test for iron is provided to demonstrate that the content of iron does not exceed the limit given in the individual monograph in terms of micrograms of iron per gram of the test substance.

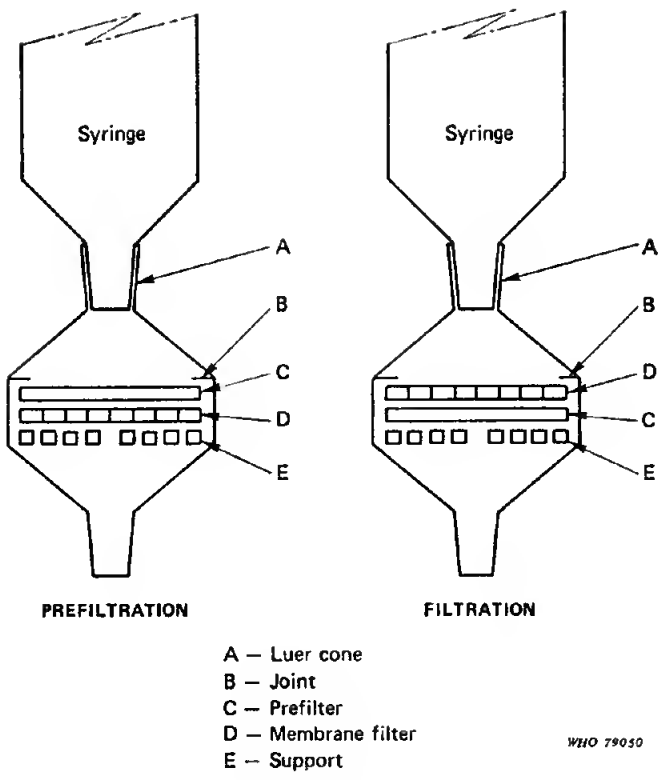


Figure 4. Limit test for heavy metals: Method B

The standard solution against which the comparison of colour is made contains 40 µg of iron.

Recommended procedure

Carry out the test in matched flat-bottomed comparison tubes of transparent glass of about 70 ml capacity and about 23 mm internal diameter bearing a 45-ml and a 50-ml mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects.

Prepare the substance as specified in the monograph, or dissolve directly an indicated quantity in 40 ml of water, and transfer to a comparison tube. Add 2 ml of citric acid (180 g/l) FeTS and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/l) FeTS, dilute to 50 ml with water, and allow to stand for 5 minutes. The colour produced is not more intense than the similarly prepared standard colour when viewed down the vertical axis of the tube in diffused light against a white background.

Standard colour
Measure 2 ml of iron standard FeTS and 40 ml of water into a comparison tube. Add 2 ml of citric acid (180 g/l) FeTS and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/l) FeTS, dilute to 50 ml with water, and allow to stand for 5 minutes.

2.2.5 Limit test for arsenic

The limit test for arsenic is provided to demonstrate that the content of arsenic does not exceed the limit given in the individual monograph in terms of micrograms of arsenic per gram of the test substance.

To carry out the limit test for arsenic a solution is prepared from the test substance by a procedure specified in the monograph. This procedure assures that the solution in every case contains the whole of the arsenic (if any) present in the substance.

The standard stain against which the comparison is made contains 10 µg of As.

The procedure described may also be used to determine the amount of arsenic in the substance by matching the depth of colour of the stain with a series of standard stains. A stain equivalent to the 1 ml standard stain produced by operating on 10 g of a substance indicates that the amount of arsenic is 1 µg/g.

In the statements of arsenic limits, the permitted amount of arsenic is expressed as As.

Apparatus
A suitable type of apparatus is described below, though other acceptable constructions are available.

A wide-mouthed bottle of about 120 ml capacity, is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm), is drawn out at one end to a diameter of about 1 mm, and has a hole not less than 2 mm in diameter blown in the side of the tube, near the constricted part. The tube is passed through the bung fitting the bottle so that, when inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded off or ground smooth.

Two rubber bungs (about 25 mm × 25 mm), each with a hole bored centrally and true and exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions of the test, as described below.

Recommended procedure

Pack the glass tube lightly with cotton-wool, previously moistened with lead acetate (80 g/l) TS and dried, so that the upper surface of the cotton-wool is not less than 25 mm below the top of the tube.

Insert the upper end of the tube into the narrow end of one of the pair of rubber bungs, either (1) to a depth of about 10 mm in the case of the tube with the rounded-off end or (2) so that the ground end of the tube is flush with the larger end of the bung. Place a piece of mercuric bromide paper AsR flat on the top of the bung, and place the other bung over it. Secure the assembly by means of a rubber band or spring clip, in such a manner that the borings of the two bungs (or the boring of the upper bung and the glass tube) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of mercuric bromide paper AsR.

Instead of this method of attaching the mercuric bromide paper AsR, any other method may be used provided (1) that the whole of the evolved gas passes through the paper, (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter, and (3) that the paper is protected from sunlight during the test.

Place the solution, prepared as specified in the monograph, in the wide-mouthed bottle, add 1 g of potassium iodide AsR and 10 g of granulated zinc AsR, and place the prepared glass tube assembly quickly into position. Allow the reaction to proceed for 40 minutes. Compare any yellow stain that is produced on the mercuric bromide paper AsR, with a standard stain, produced in a similar manner with a known quantity of dilute arsenic AsTS. Make the comparison in daylight and immediately after simultaneous preparation of the test and standard stains; the stains fade on keeping.

The most suitable temperature for carrying out the test is generally about 40 °C but, as the rate of evolution of the gas varies somewhat with different batches of granulated zinc AsR, the temperature may be adjusted to obtain a regular, but not too violent, evolution of gas. The reaction may be accelerated by placing the apparatus on a warm surface, care being taken to ensure that the mercuric bromide paper AsR remains quite dry throughout the test.

Between successive tests, the tube must be washed with hydrochloric acid (~250 g/l) AsTS, rinsed with water, and dried.

Standard stain

Prepare a solution by adding 10 ml of stannated hydrochloric acid (~250 g/l) AsTS and 1 ml of dilute arsenic AsTS, to 50 ml of water. The resulting solution, when treated as described in the general test, yields a stain on the mercuric bromide paper AsR, referred to as the standard stain.

2.3 Sulfated ash

Recommended procedure

Accurately weigh about 1 g of the substance, or the quantity specified in the monograph, into a suitable dish (usually platinum) and moisten with sulfuric acid (~1760 g/l) TS. Heat gently to remove the excess of acid and ignite at about 800 °C until all the black particles have disappeared; again moisten with sulfuric acid (~1760 g/l) TS and reignite. Add a small amount of ammonium carbonate R and ignite to constant weight.

2.4 Oxygen flask method

The oxygen flask method for the determination of halogens and sulfur in organic compounds consists of a combustion procedure followed by appropriate titrimetric determination. Combustion of the organic material in oxygen yields water-soluble inorganic products, which are determined as directed for the individual element.

Apparatus

The combustion is carried out in a suitable conical flask into the stopper of which is fused one end of a piece of platinum wire. A flask of 500 ml is used, unless otherwise specified in the monograph. Towards the other end of the wire, a piece of platinum gauze is attached to provide a means of holding the substance clear of the absorbing liquid during combustion.

Recommended procedures

CAUTION: The analyst should wear safety glasses and use a suitable safety shield between himself and the apparatus. The flask must be scrupulously clean and free from all traces of organic solvents.

Wrap the test substance in a piece of halide-free filter-paper about 5 cm long and 3 cm wide, secure the package in the platinum gauze, and insert one end of a narrow strip of filter-paper into it. Moisten the neck of the flask with water, place the specified absorbing liquid in the flask, fill the flask with oxygen, light the free end of the narrow strip of filter-paper and immediately insert the stopper. Hold the stopper firmly in place. When vigorous burning has begun, tilt the flask to prevent incompletely burned material falling into the liquid. Immediately after combustion is completed, shake the flask intermittently for 10 minutes, place a little water around the rim of the flask, carefully withdraw the stopper, and rinse the stopper, platinum wire, platinum gauze, and sides of the flask with water. Complete the analysis of this solution as specified in the monograph.

Pulverizable substances should be finely ground and thoroughly mixed before the specified quantity is weighed.

For liquids, use capsules of suitable material (e.g. methylcellulose). Place the specified quantity on about 15 mg of ashless filter-paper flock contained in one

part of a capsule of suitable size, close the capsule, inserting one end of a narrow strip of filter-paper between the two parts, and secure the capsule in the platinum gauze.

Determination of bromine and chlorine

Using the oxygen flask method described above, burn the quantity of the substance specified in the monograph. The absorbing liquid consists of 17 ml of hydrogen peroxide (~60 g/l) TS and 3 ml of water. When the process is complete, rinse the stopper, platinum wire, platinum gauze, and sides of the flask with 40 ml of water.

Add 5 drops of bromophenol blue/ethanol TS and then, by drops, sodium hydroxide (0.1 mol/l) VS until the colour changes from yellow to blue. Then add 1 ml of nitric acid (3 g/l) TS and 5 drops of diphenylcarbazone/ethanol TS as indicator, and titrate with mercuric nitrate (0.01 mol/l) VS until the solution turns light violet.

Each ml of mercuric nitrate (0.01 mol/l) VS is equivalent to 1.598 mg of Br or 0.709 mg of Cl.

Determination of fluorine

Using the oxygen flask method described, burn the quantity of the substance specified in the monograph. The absorbing liquid consists of 15 ml of water. When the process is complete, rinse the stopper, platinum wire, platinum gauze, and sides of the flask with 40 ml of water.

Add 0.6 ml of sodium alizarinsulfonate (1 g/l) TS and then, by drops, sodium hydroxide (0.1 mol/l) VS until the colour changes from pink to yellow. Add 5 ml of acetate buffer, pH 3.0, TS and titrate with thorium nitrate (0.005 mol/l) VS until the yellow colour changes to pinkish yellow.

Each ml of thorium nitrate (0.005 mol/l) VS is equivalent to 0.380 mg of F.

If a difficulty arises in observing the colour change of the indicator, a preliminary test with a solution containing known quantities of inorganic fluoride should be performed.

Determination of iodine

Using the oxygen flask method described, burn the quantity of the substance specified in the monograph. The absorbing liquid consists of 10 ml of sodium hydroxide (0.2 mol/l) VS. When the process is complete, rinse the stopper, platinum wire, platinum gauze, and sides of the flask with 25 ml of potassium acetate TS to which 15 drops of bromine TS1 are added. Then rinse with 40 ml of water and add, by drops, formic acid (~1080 g/l) TS until discoloration, 20 ml of sulfuric acid (0.05 mol/l) VS, 0.5 g of potassium iodide R, and allow to stand for 5 minutes. Titrate the liberated iodine with sodium thiosulfate (0.05 mol/l) VS, adding starch TS as indicator towards the end of the titration.

Each ml of sodium thiosulfate (0.05 mol/l) VS is equivalent to 1.06 mg of I.

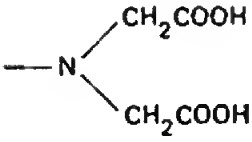
Determination of sulfur

Using the oxygen flask method described, burn the quantity of the substance specified in the monograph. The absorbing liquid consists of 12.5 ml of hydrogen peroxide (~60 g/l) TS. When the process is complete, rinse the stopper, platinum wire, platinum gauze, and sides of the flask with 40 ml of water. Boil the solution for 10 minutes, cool, add 2 ml of acetic acid (~300 g/l) TS, and 20 ml of ethanol (~750 g/l) TS. Titrate with barium nitrate (0.01 mol/l) VS using 2 drops of thorin (2 g/l) TS and 2 drops of methylthioninium chloride (0.2 g/l) TS as indicator until the yellow colour changes to pink.

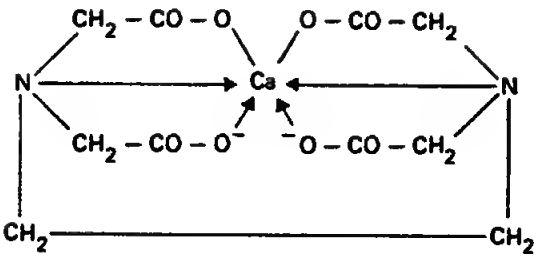
Each ml of barium nitrate (0.01 mol/l) VS is equivalent to 0.321 mg of S.

2.5 Complexometric titrations

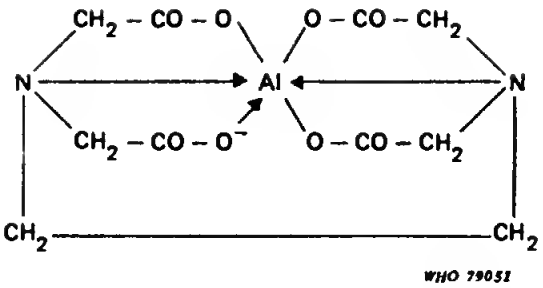
Complexing agents of value as titrants are aminopolycarboxylic acids that possess the characteristic group



Such compounds are capable of forming chelate complexes with many cations in which the cation is bound in a ring structure. The ring results from the formation of a salt-like bond between the cation and the carboxyl groups together with a coordinate bond through the lone pair of electrons of the nitrogen atom. If the ring is five-membered, the chelate thus formed is likely to have high stability, so the most useful chelating titrants are those that favour the formation of such rings. This is the case with edetic acid (ethylenediaminetetraacetic acid, EDTA); the commonly used disodium salt is known as disodium edetate. With most metals carrying more than unit positive charge, edetic acid forms highly water-soluble 1:1 complexes of such a structure that at least 3 five-membered chelate rings are formed, thus conferring high stability on the complex. In some cases, coordinate bonds other than those resulting from donation of the nitrogen lone pair of electrons may be formed with the carbonyl oxygens of the remaining carboxylic acid groups. Thus the complexes formed by calcium and by trivalent aluminium may be represented:



and



The stability of such complexes is markedly dependent on pH. Most divalent metals form complexes that are stable in alkaline solution but alkaline earth chelates decompose below about pH 8, whereas many divalent metal complexes (zinc and lead, for example) are also stable in quite acid solution. Trivalent metal complexes, by virtue of the additional stability conferred by an increased number of chelate rings, are often stable even in strongly acid solutions. In alkaline solutions, however, some of these metals may be precipitated as hydroxides in the presence of edetic acid, not because of instability of the complex but because of the more powerful effect of the very low solubility product of the metal hydroxide.

Stability constants of the edetic acid chelates of some metals, as recorded by Schwarzenbach for 0.1 mol/l solutions at 20 °C are as follows:

Na	1.7
Li	2.8
Mg	8.7
Ca	10.6
Fe^{2+}	14.3
Al	15.5[a]
Zn	16.1
Pb	17.6
Hg^{2+}	20.4
Fe^{3+}	25.1

[a] The aluminium chelate is slow to form so that this metal is usually determined by back-titration.

In order to determine the equivalence point in titration of metal ions with edetic acid, it is necessary to use a suitable indicator that will react to the presence of free metal ions in solution. The indicator originally used by Schwarzenbach for titration of calcium ions was murexide (ammonium purpurate) but this is now rarely used. Perhaps the most widely used of indicators has been Mordant Black 11 (also known under several trade names). This has a blue colour in ammoniacal solution but yields red complexes with many metal ions in such solutions; the metal complexes so formed are generally weaker than the corresponding edetic acid complexes, so that titration with edetates will readily remove the metal from the indicator complex and a colour change to full blue signifies total titration of the metal present in solution. Mordant Black 11 is fre-

quently used as a mixture with methyl orange, which serves to provide a screened (more readily detectable) end-point.

Many other substances have been proposed and used as indicators for complexometric titrations, but the present discussion must be limited to a consideration of those that are of potential value in pharmaceutical applications. Calcon and calconcarboxylic acid give a very sharp colour change from wine-red to full blue when a calcium solution is titrated with sodium edetate in the pH range 12–14. If magnesium is present it is precipitated as hydroxide at this pH and, providing the alkali is added before the indicator, does not interfere. Neither of them, however, is very stable in alkaline solution and they should preferably be added at a late stage in the titration.

Another widely used indicator is xylenol orange; this is a conventional acid-base indicator into which iminodiacetic acid groups have been introduced, thus permitting the substance to act as a metal-complexing indicator. The indicator gives a clear colour change from pink-violet to yellow at the end-point in titrations of aluminium, bismuth, lead, mercury, and zinc, and may be used from pH 2–6 according to the metal being titrated.

Recommended procedures

Aluminium

Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in 2 ml of hydrochloric acid (1 mol/l) VS and 50 ml of water, unless other conditions of solution are given in the monograph. Add 50 ml of disodium edetate (0.05 mol/l) VS and neutralize to methyl red/ethanol TS with sodium hydroxide (1 mol/l) VS. Heat the solution to boiling and maintain in a boiling water-bath for at least 10 minutes, cool, add about 50 mg of xylenol orange indicator mixture R and 5 g of methenamine R and titrate the excess edetate with lead nitrate (0.05 mol/l) VS until the yellow solution turns pink-violet. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 1.349 mg of Al.

Bismuth

Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in the minimum quantity of nitric acid (~130 g/l) TS, add 50 ml of water and adjust the pH to between 1 and 2 by dropwise addition of either nitric acid (~130 g/l) TS or ammonia (~100 g/l) TS. Add about 50 mg of xylenol orange indicator mixture R and slowly titrate with disodium edetate (0.05 mol/l) VS until the solution turns from pink-violet to a full yellow. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 10.45 mg of Bi.

Calcium

Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in a few millilitres of water, acidified with a minimum quantity of hydrochloric acid (~70 g/l) TS if necessary, and then dilute to about 100 ml with water. Titrate with disodium edetate (0.05 mol/l) VS to within about 2 ml of the

expected equivalence point, add 4 ml of sodium hydroxide (~300 g/l) TS and 0.1 g of calcon indicator mixture R or of calcon carboxylic acid indicator mixture R and continue the titration until the solution turns from pink to a full blue. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.004 mg of Ca.

Lead
Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in 5–10 ml of water, acidified with a minimum quantity of acetic acid (~300 g/l) TS if necessary, and then dilute to about 50 ml with water. Add about 50 mg of xylenol orange indicator mixture R and sufficient methenamine R (about 5 g) to turn the solution red and titrate with disodium edetate (0.05 mol/l) VS until the solution turns from deep violet to full yellow. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 10.35 mg of Pb.

Magnesium
Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in 5–10 ml of water, acidified with a minimum quantity of hydrochloric acid (~70 g/l) TS if necessary, and then dilute to about 50 ml with water. Add 10 ml of ammonium chloride buffer, pH 10.0, TS and 100 mg of Mordant Black 11 indicator mixture R and titrate with disodium edetate (0.05 mol/l) VS until the solution turns from violet to green. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 1.215 mg of Mg.

Zinc
Dissolve the quantity of substance, accurately weighed, as specified in the monograph, in 5–10 ml of water, acidified with a minimum quantity of acetic acid (~300 g/l) TS if necessary, and then dilute to about 50 ml with water. Add about 50 mg of xylenol orange indicator mixture R and sufficient methenamine R (about 5 g) to turn the solution red and titrate with disodium edetate (0.05 mol/l) VS until the solution turns from pink-violet to full yellow. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 3.268 mg of Zn.

2.6 Non-aqueous titration

Acids and bases have long been defined as substances that, when dissolved in water, furnish hydrogen and hydroxyl ions, respectively. This definition, introduced by Arrhenius, fails to recognize the fact that properties characteristic of acids or bases may also be developed in other solvents. A more generalized definition is that of Brönsted, who defined an acid as a proton donor, and a base as a proton acceptor. Even broader is the definition of Lewis, who defined an acid as any material that will accept an electron pair, a base as any material that will donate an electron pair, and neutralization as the formation of a coordination bond between an acid and a base.

The apparent strength of an acid or base is determined by the extent of its reaction with a solvent. In aqueous solution all strong acids appear equally

strong because they react with the solvent to undergo almost complete conversion to hydronium ion (H_3O^+) and the acid anion. In a weakly protophilic solvent such as acetic acid, the extent of formation of the acetonium ion ($CH_3COOH_2^+$) due to the addition of a proton provides a more sensitive differentiation of the strength of acids and shows that the order of decreasing strength for acids is perchloric, hydrobromic, sulfuric, hydrochloric, and nitric.

Acetic acid reacts incompletely with water to form hydronium ion and is, therefore, a weak acid. In contrast, it dissolves in a base such as ethylenediamine, and reacts so completely with the solvent that it behaves as a strong acid.

This so-called levelling effect is observed also for bases. In sulfuric acid almost all bases appear to be of the same strength. As the acid properties of the solvent decrease in the series sulfuric acid, acetic acid, phenol, water, pyridine and butylamine, bases dissolved in them become progressively weaker and the differences between bases are accentuated. In order of decreasing strength, strong bases of value for non-aqueous titrations are potassium methoxide, sodium methoxide, lithium methoxide, and tetrabutylammonium hydroxide.

Many water-insoluble compounds acquire enhanced acidic or basic properties when dissolved in organic solvents. Thus the choice of the appropriate solvent permits the determination of a variety of such materials by non-aqueous titration. Further, depending upon which part of a compound is physiologically active, it is often possible to titrate that part by proper selection of solvent and titrant. Pure compounds can be titrated directly, but it is often necessary to isolate the active ingredient in pharmaceutical preparations from interfering excipients and carriers.

The types of compounds that may be titrated as acids include acid halides, acid anhydrides, carboxylic acids, amino acids, enols such as barbiturates and xanthines, imides, phenols, pyrroles, and sulfonamides. The types of compounds that may be titrated as bases include amines, nitrogen-containing heterocyclic compounds, quarternary ammonium compounds, alkali salts of organic acids, alkali salts of inorganic acids, and some salts of amines. Many salts of halogen acids may be titrated in acetic acid or acetic anhydride after the addition of mercuric acetate, which removes halide ion as the unionized mercuric halide complex. In the case of hydrochlorides of weak bases not containing acetylatable groupings it is also possible to titrate in acetic anhydride without the addition of mercuric acetate and using an indicator such as malachite green or crystal violet. Titrations carried out in the presence of an excess of acetic anhydride must be applied cautiously, however, since any reaction of the anhydride with the substance being titrated may give rise to low results.

In the titration of a basic compound, a volumetric solution of perchloric acid in glacial acetic acid is usually used, although perchloric acid in dioxan may be useful in special cases. In the titration of an acidic compound, a volumetric solution of lithium methoxide in a methanol-toluene solvent is often used. For many applications it is convenient to use a solution of tetrabutylammonium

hydroxide in toluene; sodium methoxide, formerly in wide use, may often give rise to troublesome gelatinous precipitates.

Because of interference by carbon dioxide, solvents for acidic compounds must be protected from excessive exposure to the atmosphere by a suitable cover or by an inert atmosphere during the titration. A blank determination should be carried out and the volume generally should not exceed 0.01 ml of a 0.1 mol/l titrant for each ml of solvent.

The end-point may be determined visually by colour change, or potentiometrically. If the calomel reference electrode is used, it is advantageous to replace the aqueous potassium chloride solution in the salt bridge with lithium perchlorate/acetic acid TS for titrations in acidic solvents, or potassium chloride in methanol for titrations in basic solvents. It should be recognized that certain indicators in common use (crystal violet, for example) undergo a series of colour changes and, in establishing a non-aqueous titration method for a particular use, care should be taken to ensure that the colour change specified as the end-point of the titration corresponds to the maximum value of dE/dV (where E is the electromotive force and V the volume of titrant) in a potentiometric titration of the substance under consideration.

When using titrants prepared with solvents that may have a relatively high coefficient of expansion, for example, glacial acetic acid, toluene, etc., care should be taken to compensate for differences in temperature that may exist between the time the titrant is used and that at which it was standardized.

Recommended procedures

Method A (for bases and their salts)

Prepare a solution as specified in the monograph or dissolve the substance being examined in a suitable volume of glacial acetic acid R1, previously neutralized to crystal violet/acetic acid TS, warming and cooling if necessary. Alternatively the titration blank for the solvent and indicator may be established in a separate determination. When the substance is a salt of a hydrohalic acid, add 10 ml of mercuric acetate/acetic acid TS. When the end-point is determined visually by colour change, add 2–3 drops of crystal violet/acetic acid TS, and titrate with perchloric acid of the specified concentration (mol/l) to the appropriate colour change of the indicator. When a different indicator is specified in the monograph, this indicator should also be used for the neutralization of the glacial acetic acid R1, and mercuric acetate/acetic acid TS, and the standardization of the titrant.

When the equivalence point is determined potentiometrically, the indicator is omitted and neutralization of the solution and standardization of the titrant are also carried out potentiometrically. A glass electrode and a saturated calomel cell (containing potassium chloride (350 g/l) TS) as reference electrode, are used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be a minimum of transfer of liquid from one side to the other. Serious instability may result unless the connections between the potentiometer and the electrode system are in accordance with the manufacturer's instructions.

When the temperature (t_2) at which the titration is carried out differs from the temperature (t_1) at which the titrant was standardized, multiply the volume of the titrant required by $[1 + 0.001(t_1 - t_2)]$ and calculate the result of the assay from the corrected volume.

Method B (for acids)

The titrant, solvent and (in the case where the end-point is determined visually) the indicator to be used for each substance are specified in the monograph.

Protect the solution and titrant from carbon dioxide of the atmosphere throughout the determination. This may conveniently be done by replacing the air above the titration liquid with nitrogen.

Dissolve the substance being examined in a suitable volume of the solvent previously neutralized to the indicator, warming and cooling if necessary, or prepare a solution as specified in the monograph. Titrate to the appropriate colour change of the indicator. Carry out a blank determination and make any necessary corrections. The titrant is standardized using the same solvent and indicator as specified for the substance.

When the equivalence point is established potentiometrically, the indicator is omitted and neutralization of the solution and standardization of the titrant are also carried out potentiometrically.

A glass electrode and a saturated calomel reference electrode in which the aqueous potassium chloride (350 g/l) TS has been replaced by a saturated solution of potassium chloride R in methanol R are used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be a minimum of transfer of the liquid from one side to the other. Serious instability may result unless the connections between the potentiometer and the electrode system are made in accordance with the manufacturer's instruction.

2.7 Nitrite titration

Nitrite titration is a titration method used particularly for the assay of primary aromatic amines.

The apparatus usually used in an electrometric procedure for nitrite titration is composed of an open titration vessel containing two platinum electrodes connected to a suitable circuit. The electrodes should have a potential difference of 50–100 mV. The circuit should include a device for measuring current with a sensitivity of 0.1 to 1 nA, usually with an indicating needle. The titration vessel should be provided with a suitable mechanical or magnetic stirring device, or a stream of nitrogen passing through the solution may be used to mix this solution. Electrodes made of platinum wire 0.5 mm in diameter and about 20 mm long are suitable. Before each use, the electrodes should be cleaned by immersing them for a few seconds in boiling nitric acid (~1000 g/l) TS, to which about 1 mg/ml of ferric chloride R has previously been added, and then thoroughly rinsing them with water.

Recommended procedure

Place 20 ml of hydrochloric acid (~250 g/l) TS and 50 ml of water in the titration vessel, add the quantity of the test substance and, if indicated, a catalyst, as specified in the monograph and stir to dissolve; cool to about 15 °C and titrate slowly with sodium nitrite (0.1 mol/l) VS, placing the burette tip below the surface of the solution. During the addition of the titrant, stir the solution continuously and gently, without pulling a vortex of air under the surface, and maintain the temperature of the solution at about 15 °C.

When the titration is within 1 ml of the estimated end-point, add the titrant in 0.1 ml portions, allowing not less than 1 minute to elapse before adding subsequent portions. Initially, the needle of the measuring device deflects at every addition of reagent and then returns to its original position. No deflection is observed when the end-point of the determination is reached.

2.8 Determination of water by the Karl Fischer method

The titrimetric determination of water by the Karl Fischer method depends on the reaction that takes place quantitatively between water and a reagent consisting of sulfur dioxide and iodine in anhydrous pyridine and usually methanol. The reaction is carried out in a suitable solvent such as methanol or acetic acid.

The reagents and solutions used in the determination of water by this method are sensitive to water and precautions must be taken throughout to prevent exposure to atmospheric moisture.

The titration vessel is fitted with two platinum electrodes, a gas inlet tube if needed, a stopper, which accommodates the burette tip, and a vent tube protected by a desiccant. The substance to be titrated is introduced through an inlet tube or side-arm, which can be closed by an airtight stopper. The Karl Fischer reagent TS is protected from light and stored in a bottle into which is fitted an automatic burette. The reagent is pumped into the burette by means of a hand bellows, the access of moisture being prevented by a suitable arrangement of desiccant tubes. Stirring is accomplished magnetically or by means of a stream of suitably dried nitrogen passed through the solution during the titration.

The end-point is obtained by using an electrical circuit composed of a microammeter, platinum electrodes, and a 1.5-V or 2-V battery connected across a variable resistance of about 2000 Ω. The resistance is adjusted so that an initial current passes through the platinum electrodes in series with a microammeter. After each addition of reagent, the pointer of the microammeter is deflected but quickly returns to its original position. At the end of the reaction a deflection is obtained that persists for 10–15 seconds. Alternatively, the end-point can also be determined by a voltametric method. A potential difference of 30–50 mV is applied to the platinum electrodes to serve as a constant polarizing current and the solution is titrated with the reagent. The potential difference is monitored by means of a microvoltmeter. The end-point is reached when the voltmeter indicates a stable decrease of voltage. In the voltametric method the end-point may also be obtained graphically by plotting the voltage

versus the volume of the reagent, and establishing the beginning of the drop in potential.

Recommended procedures

Direct titration (Method A)

Add about 20 ml of dehydrated methanol R, unless otherwise specified in the monograph, to the titration vessel and titrate to the end-point with Karl Fischer reagent TS. Quickly transfer the specified quantity of substance, accurately weighed, to the titration vessel. Stir for 1 minute and titrate again to the end-point with Karl Fischer reagent TS.

Backtitration (Method B)

Add about 10 ml of dehydrated methanol R, unless otherwise specified in the monograph, to the titration vessel and titrate to the end-point with Karl Fischer reagent TS. Quickly transfer the specified quantity of substance, accurately weighed, to the titration vessel, followed by an accurately measured amount of Karl Fischer reagent TS, sufficient to give an excess of about 1 ml. Allow to stand protected from light for 1 minute or the time specified in the monograph, stirring from time to time.

Titrate the excess of Karl Fischer reagent TS to the end-point with dehydrated methanol R, to which has been added an accurately known amount of water, usually equivalent to about 2.5 mg/ml.

2.9 Determination of methoxyl

The contents of methoxy-groups in an organic substance are assayed by reacting the substance with concentrated hydriodic acid; methyl iodide produced in the reaction is distilled off, absorbed into a suitable absorbing liquid, and its amount determined titrimetrically.

Apparatus

The apparatus consists of a 25-ml round-bottomed boiling flask into which is sealed a capillary side-arm, 1 mm in diameter, to provide an inlet for a stream of carbon dioxide. The flask is also fitted with an air condenser, approximately 25 cm in height and about 9 mm in internal diameter. A suitable scrubber device containing about 2 ml of water is placed over the condenser. Add 5 ml of antimony sodium tartrate (50 g/l) TS to the scrubber. The outlet from the scrubber terminates in a tube that dips below the surface of the absorbing liquid in the first of two receivers connected in series. For greater convenience in use and cleaning, separate parts of the apparatus are connected by means of ground glass conical or ball joints.

Recommended procedure

Place a quantity of the substance being tested, accurately weighed, as specified in the monograph, in the boiling flask with a boiling rod. Add 2.5 ml of melted phenol R and 5 ml of hydriodic acid (~970 g/l) TS, and connect the flask to the condenser. Add potassium acetate TS to each of the two receivers, about 6 ml to the first one and about 4 ml to the second, and to each receiver 6 drops of bromine R. Pass a slow uniform stream of carbon dioxide R through the capillary side-arm of the boiling flask, and heat the liquid gently by means of a mantled microburner or another suitable device, at such a rate that the vapours of the boiling liquid rise halfway up the condenser. For most substances 30 minutes are sufficient to complete the reaction and sweep out the apparatus. Wash the contents of both receivers into a 250-ml conical flask containing 5 ml of sodium acetate (150 g/l) TS.

Adjust the volume of the liquid to approximately 125 ml, and add 6 drops of formic acid (~1080 g/l) TS. Rotate the flask until the colour due to the bromine is discharged, then add 12 drops of formic acid (~1080 g/l) TS, stopper the flask and mix the contents thoroughly so as to remove any free bromine including the vapour above the liquid, and allow the solution to stand for 1–2 minutes; add 1 g of potassium iodide R and 5 ml of sulfuric acid (~100 g/l) TS, and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS using starch TS as indicator. Repeat the operation without the substance being tested, and deduct the volume of sodium thiosulfate (0.1 mol/l) VS used from the volume required in the determination of methoxyl.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 0.5172 mg of methoxyl (CH_3O).

2.10 Determination of nitrogen

Recommended procedures

Procedure for macrodetermination (Method A)

Place the amount of test substance specified in the monograph in a 200-ml long-necked flask, add 1 g of a mixture composed of 10 parts of potassium sulfate R or anhydrous sodium sulfate R and 1 part of copper(II) sulfate R, followed by nitrogen-free sulfuric acid (~1760 g/l) TS, using the quantities of the substance and of sulfuric acid specified in the monograph. Heat the mixture over a small flame until a clear green solution is obtained and boil gently for a further 30 minutes unless otherwise specified in the monograph; precautions should be taken to prevent the upper part of the flask from becoming overheated. Cool, dilute to 75–85 ml with water, using suitable precautions, and add a piece of granulated zinc R and a solution of 15 g of sodium hydroxide R and 2 g of sodium thiosulfate R in 25 ml of water. The quantity of sodium hydroxide should be increased, if necessary, to ensure that, before distillation, the mixture is strongly alkaline. Immediately connect the flask to a distillation apparatus, mix the contents, distil the liberated ammonia into 16 ml of boric acid (50 g/l) TS, and titrate with sulfuric acid (0.05 mol/l) VS using methyl red/ethanol TS

as indicator. Repeat the operation without the substance being tested; the difference between the titrations represents the ammonia liberated by the substance being tested. Each ml of sulfuric acid (0.05mol/l) VS is equivalent to 1.401mg of N.

Procedure for microdetermination (Method B)
Place the amount of test substance specified in the monograph in a suitable digestion tube, add 3 drops of copper(II) sulfate (190g/l) TS and 1ml of nitrogen-free sulfuric acid (~1760g/l) TS and boil gently for 10 minutes; cool; add 1g of anhydrous sodium sulfate R and 10mg of selenium R, boil gently for 1 hour and cool. Transfer the contents of the digestion tube to (or attach it to) an ammonia microdistillation apparatus, add 6ml of sodium hydroxide (~400g/l) TS and pass steam through the flask; distil for 7 minutes, collecting the distillate in a mixture of 5ml of boric acid (50g/l) TS, 5ml of water, and 1 drop of methyl red/methylthioninium chloride TS and titrate with hydrochloric acid (0.015mol/l) VS. Repeat the operation without the substance being tested; the difference between the titrations represents the ammonia liberated by the substance being tested. Each ml of hydrochloric acid (0.015mol/l) VS is equivalent to 0.210mg of N.

3. Biological methods

3.1 Microbiological assay of antibiotics

The potency (activity) of an antibiotic product is expressed as the ratio of the dose that inhibits the growth of a suitable susceptible microorganism to the dose of an International Biological Standard, an International Biological Reference Preparation, or an International Chemical Reference Substance of that antibiotic that produces similar inhibition. Properly validated secondary reference materials may also be used in the assay. To carry out the assay a comparison is made between the inhibition of the growth of microorganisms produced by known concentrations of the reference material and that produced by measured dilutions of the test substance. This response can be measured by the diffusion method, as described below, or in a liquid medium by the turbidimetric method.

An International Unit is the specific activity contained in such an amount (weight) of the relevant International Biological Standard or International Biological Reference Preparation as the WHO Expert Committee on Biological Standardization may from time to indicate as the quantity exactly equivalent to the unit accepted for international use. In some cases, when owing to the properties of the material, difficulties are experienced in weighing with adequate accuracy small amounts of the relevant International Biological Standard or International Biological Reference Preparation, International Units are defined on the basis of the total contents of the material in an ampoule or a vial. A defined number of International Units is then assigned to the total contents of

an ampoule or a vial; this material has to be carefully removed with an appropriate solvent and the final volume of the solution has to be accurately adjusted.

International Chemical Reference Substances do not have defined units of biological activity. The potency of those products for which biological assays are required are in such cases expressed in terms of an equivalent weight of the pure substance.

Recommended procedure

Use Petri dishes or rectangular trays filled to a depth of 3–4 mm, unless otherwise indicated in the monograph, with a culture medium that has previously been inoculated with a suitable inoculum of a susceptible test organism prepared as described below. The nutrient agar may be composed of two separate layers of which only the upper one may be inoculated. The concentration of the inoculum should be so selected that the sharpest zones of inhibition and suitable dose response at different concentrations of the standard are obtained. When using the inoculum prepared as described below, an inoculated medium containing 1 ml of inoculum per 100 ml of the culture medium is usually suitable. When the inoculum consists of a suspension of vegetative organisms, the temperature of the molten agar medium must not exceed 48–50 °C at the time of inoculation. The dishes or trays should be specially selected with flat bottoms. During the filling they should be placed on a flat, horizontal surface so as to ensure that the layer of the medium will be of a uniform thickness. With some test organisms, the procedure may be improved if the inoculated plates are allowed to dry for 30 minutes at room temperature before use, or refrigerated at 4 °C for several hours.

For the application of the test solution, small sterile cylinders of uniform size, approximately 10 mm high and having an internal diameter of approximately 5 mm, made of suitable material such as glass, porcelain, or stainless steel, are placed on the surface of the inoculated medium. Instead of cylinders, holes 8–10 mm in diameter may be bored in the medium with a previously sterilized borer. Other methods of application of the test solution may also be used. The arrangement on the plate should be such that overlapping of zones is avoided.

Solutions of the reference material of known concentration and corresponding dilutions of the test substance, presumed to be of approximately the same concentration, are prepared in a sterile buffer of a suitable pH value. To assess the validity of the assay at least 3 different doses of the reference material should be used together with an equal number of doses of the test substance having the same presumed activity as the solutions of the reference material. The dose levels used should be in geometric progression, for example, by preparing a series of dilutions in the ratio 2:1. Once the relationship between the logarithm of concentration of the antibiotic and the diameter of the zone of inhibition has been shown to be approximately rectilinear for the system used, routine assays may be carried out using only 2 concentrations of the reference material and 2 dilutions of the test substance. Where a mono-

graph gives directions for the initial preparation of a solution of the substance, this solution is then diluted as necessary with the appropriate sterile buffer.

The solutions of the reference material and the test substance are preferably arranged in the form of a Latin square when rectangular trays are employed. When Petri dishes are used, the solutions are arranged on each dish so that the solutions of the reference material and those of the test substance alternate around the dish and are placed in such a manner that the highest concentrations of the reference material and of the test substance are not adjacent. The solutions are placed in the cylinders or holes by means of a pipette that delivers a uniform volume of liquid. When the holes are used the delivered volume should be sufficient to fill them almost completely.

The plates are incubated at a suitable temperature, the selected temperature being controlled at ±0.5 °C, for approximately 16 hours, and the diameters or areas of the zones of inhibition produced by the varied concentrations of the standard and of the test substance are measured accurately, preferably to the nearest 0.1 mm of the actual zone size, by using a suitable measuring device. From the results, the potency of the tested substance is calculated. Suitable publications on the statistics of bioassays are listed below.

Conditions for the assay of individual antibiotics and suitable test organisms are given in the monographs. The choice of an appropriate strain of test organism may be critical for the assay. For easy reference, examples of suitable test organisms for a number of antibiotics are shown in Table 4. The designations of the test strains are as follows:

NCTC – National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England
NCYC – National Collection of Yeast Cultures, AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA, England
ATCC – American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA

Other suitable strains of test organisms can be used. Additional information regarding sources of suitable strains may be obtained from Quality Assurance and Safety: Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

Precision of the assay

In order to determine whether or not a substance satisfies the requirements for potency specified in the monograph, the assay should, if necessary, be repeated until the required precision has been attained. This precision is such that the fiducial limits (P = 0.95) of the mean estimated potency, expressed as a percentage of the mean estimated potency, should be within the required range given in the individual monographs.

Calculation of results

The following publications contain suitable methods that may be used to carry out the statistical evaluation of the microbiological assay of antibiotics:

1. Bliss CI. *Statistics of bioassay*. New York, Academic Press, 1952.
2. Bliss CI. *Statistics in biology*, vol. I. New York, McGraw Hill, 1967.
3. Bliss CI. *Statistics in biology*, vol. II. New York, McGraw Hill, 1970.
4. Finney DJ. *Statistical methods in biological assays*. London, Griffin, 1964.
5. Hewitt W. *Microbiological assay*. New York, Academic Press, 1977.
6. Philippe J. *Les methodes statistiques en pharmacie et en chimie*. Paris, Masson, 1967.

The methods of carrying out the statistical evaluation of the microbiological assay of antibiotics are also described in many national and regional pharmacopoeias.

Culture media

The formulae for the culture media (Cm) referred to in Table 4 are described in Reagents, test solutions, and volumetric solutions. In each instance the final pH is adjusted to that stated in the table.

Preparation of inoculum

Bacillus cereus; Bacillus pumilus; Bacillus subtilis. The test organism is grown for 7 days at a temperature of 37–39 °C on the surface of culture medium Cm1 (pH 6.5–6.6 after sterilization) to which has been added 1 µg of manganese sulfate R per ml. Using sterile water, the growth, which consists mainly of spores, is washed off, heated for 30 minutes at 70 °C, and suitably diluted – for example, to give between 10^7 and 10^8 spores per ml. The spore suspension may be stored for long periods at a temperature not exceeding 4 °C.

Bordetella bronchiseptica. The test organism is grown overnight on culture medium Cm2 (pH 6.5–6.6 after sterilization) at a temperature of 35–37 °C. A suspension is prepared by washing off the growth and diluting with sterile water or saline TS to a suitable opacity, for example, such that a 1-cm layer transmits 50% of the incident light when examined at 650 nm. The suspension may be stored for up to 2 weeks at a temperature not exceeding 4 °C.

A freshly prepared inoculum may be replaced by a suitable suspension of the inoculum in a suitable vehicle, such as sterile peptone (1 g/l) TS2 that has been stored frozen at –70 °C and subsequently thawed.

Micrococcus luteus. The test organism is grown overnight on culture medium Cm1 (pH 6.5–6.6 after sterilization) at a temperature of 35–37 °C. A suspension is prepared by washing off the growth and diluting with saline TS to a suitable opacity, for example, such that a 1-cm layer of a 1 in 50 dilution transmits 80% of the incident light when examined at 650 nm. The suspension may be stored for up to 2 weeks at a temperature not exceeding 4 °C.

A freshly prepared inoculum may be replaced by a suitable suspension of the inoculum in a suitable vehicle, such as sterile peptone (1 g/l) TS2 that has been stored frozen at –70 °C and subsequently thawed.

Table 4. Test organisms and conditions of assay of individual antibiotics

Antibiotic	Test organism	Culture medium; final pH	Phosphate buffer, sterile, pH[a], TS	Concentration (weight or International Units per ml)[b]	Incubation temperature (in °C)
Bacitracin	*Micrococcus luteus* NCTC 7743; ATCC 10240	Cm1; 7.0–7.1	7.0	1–4 IU	35–37
	Micrococcus luteus NCTC 7743; ATCC 10240	Cm1; 6.5–6.6	6.0	1–4 IU	30–31
Cefalexin	*Staphylococcus aureus* NCTC 6571; ATCC 9144	Cm1; 6.5–6.6	6.0	10–40 µg	32–35
	Staphylococcus aureus ATCC 6538-P	Cm1; 6.5–6.6	6.0	10–40 µg	32–35
Cefalotin	*Staphylococcus aureus* NCTC 6571; ATCC 9144	Cm1; 6.5–6.6	6.0	0.5–2 IU	32–35
	Staphylococcus aureus ATCC 6538-P	Cm1; 6.5–6.6	6.0	0.5–2 IU	32–35
Chlortetracycline	*Bacillus pumilus* NCTC 8241; ATCC 14884	Cm1; 6.5–6.6	4.5	2–20 IU	37–39
	Bacillus cereus ATCC 11778	Cm1; 5.9–6.0	4.5	0.05–0.2 IU	30–33
Cloxacillin	*Bacillus subtilis* NCTC 8236; ATCC 11774	Cm1; 6.5–6.6	7.0	5–20 µg	37–39
	Staphylococcus aureus ATCC 6538-P	Cm1; 6.5–6.6	6.0	2–8 µg	32–35
Dicloxacillin	*Staphylococcus aureus* NCTC 6571; ATCC 9144	Cm1; 6.6	6.0	2.5–10 µg	37–39
	Staphylococcus aureus ATCC 6538-P	Cm1; 6.5–6.6	6.0	2–8 µg	32–35
Erythromycin	*Bacillus pumilus* NCTC 8241; ATCC 14884	Cm1; 8.0–8.1	8.0	5–25 IU	37–39
	Micrococcus luteus ATCC 9341	Cm1; 8.0–8.1	8.0	0.5–1.5 IU	35–37
Neomycin	*Bacillus pumilus* NCTC 8241; ATCC 14884	Cm1; 8.0–8.1	8.0	2–14 IU	37–39
	Staphylococcus aureus ATCC 29737	Cm1; 7.8–8.0	8.0	2–20 IU	35–37
	Staphylococcus epidermidis ATCC 12228	Cm1; 8.0–8.1	8.0	0.5–2 IU	35–37
Novobiocin	*Bacillus subtilis* NCTC 10315;	Cm1; 6.5–6.6	6.0	1–5 IU	30–33

Table 4. Continued

Antibiotic	Test organism	Culture medium; final pH	Phosphate buffer, sterile, pH[a], TS	Concentration (weight or International Units per ml)[b]	Incubation temperature (in °C)
	Micrococcus luteus ATCC 9341	Cm1; 6.5–6.6	6.0	10–50 IU	30–35
Nystatin	*Saccharomyces cerevisiae* NCYC 87; ATCC 9763	Cm3; 6.0–6.2	-[c]	25–300 IU	35–37
Oxacillin	*Bacillus subtilis* NCTC 8236; ATCC 11774	Cm1; 6.5–6.6	7.0	2.5–10 µg	37–39
	Staphylococcus aureus ATCC 6538-P	Cm1; 6.5–6.6	6.0	2–8 µg	32–35
Oxytetracycline	*Bacillus pumilus* NCTC 8241; ATCC 14884	Cm1; 6.5–6.6	4.5	2–20 IU	37–39
	Bacillus cereus ATCC 11778	Cm1; 5.9–6.0	4.5	0.5–2 IU	30–33
Polymyxin B	*Bordetella bronchiseptica* NCTC 8344; ATCC 4617	Cm2; 7.2–7.3	6.0, TS3	20–100 IU	35–37
	Bordetella bronchiseptica NCTC 8344; ATCC 4617	Cm2; 7.2–7.3	7.2	50–200 IU	35–37
	Escherichia coli ATCC 10536	Cm1; 6.5–6.6	7.2	5–100 IU	35–37
Streptomycin	*Bacillus subtilis* NCTC 8236; ATCC 11774	Cm1; 7.9–8.0	8.0	5–20 IU	37–39
	Bacillus subtilis ATCC 6633	Cm1; 8.0–8.1	8.0	3–15 IU	35–37
Tetracycline	*Bacillus pumilus* NCTC 8241; ATCC 14884	Cm1; 6.5–6.6	4.5	2–20 IU	37–39
	Bacillus cereus ATCC 11778	Cm1; 5.9–6.0	4.5	0.5–2 IU	30–33

[a] Phosphate buffers, sterile, of suitable pH. Buffers designated as TS, TS1, or TS2 may be used.
[b] Range within which suitable concentrations may be found.
[c] The preparation of the solution of the reference material and of the corresponding dilution of the test substance is done as described in the monograph with the aid of dimethylformamide R and phosphate buffer, sterile, pH 6.0 TS3.

Saccharomyces cerevisiae. The test organism is grown overnight on culture medium Cm3 (pH 6.0–6.2 after sterilization) at a temperature of 35–37 °C. A suspension is prepared by washing off the growth with saline TS and diluting to a suitable opacity, for example, such that a 1-cm layer transmits 50% of the incident light when examined at 650 nm. The suspension may be stored for up to 2 weeks at a temperature not exceeding 4 °C.

A freshly prepared inoculum may be replaced by a suitable suspension of the inoculum in a suitable vehicle, such as sterile peptone (1 g/l) TS2 that has been stored frozen at –70 °C and subsequently thawed.

Staphylococcus aureus. The test organism is grown overnight on culture medium Cm1 (pH 6.5–6.6 after sterilization) at a temperature of 35–37 °C. A suspension is prepared by washing off the growth with saline TS and diluting to a suitable opacity, for example, such that a 1-cm layer transmits 50% of the incident light when examined at 650 nm.

A freshly prepared inoculum may be replaced by a suitable suspension of the inoculum in a suitable vehicle, such as sterile peptone (5 g/l) TS that has been stored frozen at –70 °C and subsequently thawed.

3.2 Test for sterility

This test for sterility is applicable only to parenteral preparations. For ophthalmic and other non-injectable preparations, surgical dressings, catgut and other surgical sutures, appropriate alternative test conditions must be chosen.

Sterility testing should be carried out under strict conditions specifically designed to avoid microbial contamination of the material being tested, for example by using a laminar flow technique. Precautions should also be taken to ensure that any microorganisms expected to be revealed by the test remain unmodified. Adequate performance of the testing technique should be ensured periodically.

A batch (i.e. one sterilized load, or a quantity of containers filled under aseptic conditions in one working session at one work station) is defined as a collection of sealed containers whose contents and physical aspect are homogeneous in every respect. The risk of microbial contamination is thus assumed to be equal for each container in the batch.

Therefore, once a sample has been tested and is found to be free of contamination, the same degree of purity should apply to the whole batch. Assurance that this is the case depends strongly on compliance with Good Manufacturing Practices (GMP)[1] throughout the entire manufacturing process.

[1] WHO good manufacturing practices: main principles for pharmaceutical products. Quality assurance of pharmaceuticals. A compendium of guidelines and related materials, Volume 2, Updated edition. Geneva, World Health Organization, 2004. However, many national authorities have incorporated good manufacturing practices of their own into their legislation, and these should be followed when available.

Sampling

Number of containers in the batch	Minimum number of samples to be tested
not more than 100	10% or 4 containers (whichever is greater)
between 100 and 500	10 containers
more than 500	2% or 20 containers (whichever is less)

Culture media

Culture media that have proven to be the most suitable for use in sterility tests are: *fluid sodium mercaptoacetate* and *soya-bean casein digest media. Fluid sodium mercaptoacetate* (sodium thioglycolate) *medium* (culture medium Cm4) is primarily intended for the culture of anaerobic bacteria but will also detect aerobic bacteria. *Soya-bean casein digest medium* (culture medium Cm5) is primarily intended for the culture of aerobic bacteria but is also suitable for fungi. Other media may be used provided that they have been shown to sustain the growth of a wide range of microorganisms and that they comply with the test for "effectiveness of the medium" in the presence of the preparation to be tested.

The growth-promoting quality of each new batch of media should be tested as follows: take separate representative samples of the medium and add inocula of reference strains of appropriate microorganisms (e.g. *Staphylococcus aureus* ATCC 6538 P (NCIMB 8625, CIP 53.156), *Bacillus subtilis* ATCC 6633, *Clostridium sporogenes* ATCC 19404, *Candida albicans* ATCC 2091), each containing approximately 100 viable microorganisms. The media thus inoculated with the reference strains should then be incubated according to the conditions specified in the recommended procedure. The test media are satisfactory if clear evidence of growth appears in all inoculated media within 7 days.

Antibacterial effects of the sample

Before testing, possible antibacterial action of the substance or material under examination should be determined and the growth-promoting properties of the media in the presence and in the absence of the test sample should be compared. This comparison should be carried out under test conditions using reference strains, as described above in the section "Culture media". If the growth of the reference strains is delayed or weakened in the presence of the product, the antibacterial action of the product should be suppressed by means of filtration, dilution, or neutralization. The effectiveness of this process should be confirmed by repeating the test.

Recommended procedures

The test for sterility may be carried out by membrane filtration or by direct inoculation. The technique of membrane filtration is to be preferred whenever the nature of the product permits, i.e. for aqueous preparations able to be filtered, especially in large volumes, for alcoholic or oily preparations, and for

preparations miscible with or soluble in aqueous or oily solvents that have not shown any antimicrobial effect when tested. Whichever test is used, the media should be observed at regular intervals throughout the specified incubation period.

Membrane filtration

Use membrane filters of assured quality, preferably certified by the manufacturer, with a nominal pore size not greater than 0.45 µm. For example, use cellulose nitrate filters for aqueous, oily, and weakly alcoholic liquids and acetate filters for strongly alcoholic liquids. Use filter discs with a diameter of about 50 mm in a previously sterilized filter assembly. If filters of a different diameter are used, adjust the volumes of the dilutions and the washings accordingly.

Before the test, filter a small quantity of a suitable sterile diluent, such as a 1 g/litre neutral solution of meat or casein peptone (culture media Cm1 or Cm5), through the prepared filtration apparatus. Transfer the contents of the container(s) to be examined to the apparatus. If possible, transfer the entire contents of the containers or the minimum quantity laid down for the test by direct inoculation. If necessary, dilute the product to be tested with the chosen sterile diluent. Carry out the filtration without delay.

If the product to be tested has known antimicrobial properties, wash the membrane by filtering through it not less than three successive quantities of the chosen sterile diluent, each of approximately 100 ml, with the addition of a suitable neutralizing substance. Oily liquids may be diluted before filtration with a suitable sterile solvent, such as isopropyl myristate, shown to have minimal antimicrobial effects.

Preferably, transfer one membrane to each of the media used or, where this is not possible, transfer the one membrane cut aseptically into two equal parts to the different media. Incubate the culture media with the membranes for not less than 7 days at 30–35 °C if it is intended mainly to detect bacteria and at 20–25 °C if it is specifically intended to detect fungi. If the product or the treatment to which it has been subjected is suspect, a longer incubation time than 7 days may be necessary.

Direct inoculation

Transfer directly a suitable quantity (see the following tables) of the product under examination, taken from a sealed container, to culture media intended for the detection of aerobic and anaerobic bacteria as well as media for the detection of fungi.

Liquids

Quantity in the container	Quantity of sample needed
less than 1 ml	entire contents of container
between 1 ml and 4 ml	half contents of container
between 4 ml and 20 ml	2 ml
20 ml or more (including large-volume parenterals)	10% of contents

Solids

Quantity in the container	Quantity of sample needed
less than 50 mg	entire contents of container
between 50 mg and 200 mg	50% of contents of container
200 mg or more	100 mg

There should be a sufficient quantity of culture medium to ensure that its nutritive properties are unaffected by the addition of the product under examination. In order to ensure homogeneous distribution and to eliminate antibacterial activity, unless otherwise indicated in the monograph, transfer the product under examination in such a way that liquids are diluted approximately 10-fold and solids approximately 100-fold.

In the case of an oily liquid, an emulsifying agent may be added to the culture medium, such as 0.5–1% *m/v* of polysorbate 80 or 0.1% *m/v* of (*p-tert*-octylphenoxy)polyoxyethanol. Other emulsifying agents shown not to have any antimicrobial action under test conditions may also be used in appropriate concentrations.

Incubate the inoculated media for not less than 14 days. Maintain the temperature at 30–35 °C for media mainly intended to detect bacteria and at 20–25 °C for media specifically intended to detect fungi. Gently shake at regular intervals if the medium contains an oily liquid. If one medium is used to detect both aerobic and anaerobic bacteria, keep the shaking to a minimum in order to maintain anaerobic conditions.

Interpretation of results

If no microbial growth appears in any of the culture media by the end of the incubation period, the product complies with the test for sterility.

If microbial growth appears in the initial test cultures and/or in subcultures, the organisms should be isolated and identified. A second test should then be made to verify the results.

- If there is evidence – from the material used, the kind of organisms detected, the monitoring or the control tests – that the test was carried out

under inadequately aseptic conditions, the test is invalid and should be repeated with the same corresponding number of items and quantities.

- If the microbial growth is not due to lack of aseptic conditions, the test should be repeated on twice the number of containers used for the original test. If no microbial growth is detected, the product examined complies with the test for sterility. If microbial growth is detected in the second test, the product examined does not comply with the test for sterility.

3.2.1 Test for sterility of non-injectable preparations

The methods are described under 3.2 Test for sterility.

Certain dosage forms such as ophthalmic drops, ophthalmic ointments, topical semi-solid dosage forms to be applied to damaged skin, etc., require specific sampling plans and preparation of samples.

For the membrane filtration method and for direct inoculation of the medium, determine the number of containers according to the sampling method proposed. The quantity taken should be not less than the minimum and not more than the maximum quantity given below. The quantity used for each medium, which should be taken from the mixed sample, is also specified in the table.

Sampling plan

Type of preparation	Total pooled for each medium	Quantity to be used
	Membrane filtration method	
Aqueous solution	10–100 ml	5–10 ml
Preparations soluble in water, isopropyl myristate, or other solvents	1–10 g	equivalent to 0.5–1 g
	Direct inoculation method	
Liquid preparations	10–100 ml	5–10 ml
Soluble preparations	1–10 g	equivalent to 0.5–1 g
Insoluble preparations to be suspended or emulsified, e.g. creams and ointments	1–10 g	equivalent to 0.5–1 g

If the membrane filtration method is used for ointments and creams, the preparation being tested may require additional heating up to 40 °C (or exceptionally up to 45 °C with continuous stirring). The filtration process should be carried out as quickly as possible. If heat is applied and/or a solvent combination is used in the sample preparation, the method should first be validated to ensure that the specific expected bioburden is not affected by these conditions.

3.2.2 Sterility testing of antibiotics

The test is designed to reveal the presence of contamination with live microorganisms in antibiotics intended for parenteral administration or for other sterile applications.

Test conditions

The test should be carried out under aseptic conditions in an area as free from contamination as it is possible to achieve by the use of disinfecting agents, germicidal lamps, and air filters. Germicidal lamps and disinfecting aerosols should not be used during actual testing operations. The test manipulations should be carried out in a filtered air environment or under a laminar flow hood, with operators dressed in sterilized, static-free clothing, including head and foot wear. The air pressure in the testing room should be greater than that of the exterior area. The performance of the laminar flow hood should be monitored by particulate count, settle plates, or slit-sampling devices, and the performance of the filters and germicidal lamps checked routinely.

Membrane filtration apparatus

A suitable unit consists of a closed reservoir and a receptacle, separated by a properly supported membrane of appropriate porosity. Membranes generally suitable for sterility testing have a nominal porosity of 0.45 µm, a diameter of approximately 47 mm, and a flow rate of 55–75 ml of water per minute at a pressure of 90 kPa (700 mm Hg). The entire unit should preferably be assembled and sterilized with the membrane in place, prior to use. If each entire membrane is to be cultured, at least 2 filter units should be set up.

All air entering the filtering unit should be passed through an air-filter capable of removing microorganisms.

Sampling

Take the sample in such a manner as to be representative of the material to be tested. The amount taken should be sufficient to perform the tests and any repeat tests that may be required. The sampling should be carried out in such a way as to maintain intact the sterility of the material.

Culture media

The culture media used for sterility tests for bacteria and fungi should be capable of supporting the growth of a wide variety of microorganisms, with both aerobic and anaerobic growth characteristics, including the types found in the environment of the manufacturing operations. More than one culture medium will generally be needed to fulfil these criteria. The media that usually give satisfactory results are fluid mercaptoacetate (thioglycolate) medium (culture medium Cm4) and soybean-casein digest medium (culture medium Cm5). Any other media that are used, however, should have at least demonstrably equivalent growth-supporting properties.

For testing the growth-supporting properties of each culture medium, strains of microorganisms should be used with exacting nutritive and aerobic-anaerobic requirements, in an inoculum containing only a small number of organisms (less than 100). The media should be incubated at the temperatures at which they will be used in the sterility test and growth should be evident after 24 hours.

Each lot of dehydrated medium obtained from a specialized manufacturer or each lot of the medium prepared entirely in the laboratory should be tested for its growth-supporting properties, since not every lot may support the growth of microorganisms to the desired extent. Differences may be caused by the occasional presence of unsatisfactory components in a particular lot, or by the destruction of certain components by overheating or oversterilization of the medium.

Recommended procedures

Membrane filtration test procedure

Aseptically transfer a suitable amount of the solid test material (0.3–6 g depending on the size of the container) into a sterile flask containing about 200 ml of peptone (1 g/l) TS1, stopper the flask and swirl to effect rapid dissolution. If the test material dissolves slowly or if the resulting solution will not filter rapidly, the volume of the solvent may be increased to not more than 400 ml. Immediately after the test material has dissolved, aseptically filter the solution, with the aid of reduced pressure, through the membrane filter previously moistened with sterile water or with peptone (1 g/l) TS1. To speed up the process of filtration, the solution may be filtered using two filtering units simultaneously. To remove the residual antibiotic from the membrane, wash it with sufficient peptone (1 g/l) TS1 to which, if so indicated in the monograph (in the case of penicillin and cephalosporin antibiotics), sufficient penicillinase TS has been added.

Upon completion of the filtration, divide the membrane aseptically into two approximately equal parts. Transfer one part of the membrane into a culture vessel (a test-tube is suitable) containing 50–100 ml of culture medium Cm4 (fluid mercaptoacetate (thioglycolate) medium) and the other part of the membrane into another culture vessel containing 50–100 ml of culture medium Cm5 (soybean-casein digest medium).

A control test should be carried out at appropriate intervals to demonstrate that residual antibiotic activity is being reduced by the above described washing procedure to below the level that allows growth of an inoculum containing 50–100 microorganisms susceptible to the antibiotic tested.

Direct test procedure

Depending on the size of the container take from 1–10 portions, each 0.3 g of the test material, and aseptically transfer them into individual sterile vessels (test-tubes are suitable) containing 50–100 ml of culture medium Cm6 (fluid mercaptoacetate (thioglycolate) medium with penicillinase). Transfer portions of similar size to another set of individual sterile vessels containing 50–100 ml of culture medium Cm7 (soybean-casein digest medium with penicillinase).

Check the ability of the penicillinase contained in the medium to inactivate all the penicillin in the test material by adding to one vessel containing culture medium Cm6 the amount of material taken from one container under test. Next add 1.0 ml of a dilution containing 50–100 microorganisms of a suitable strain (*Staphylococcus aureus,* ATCC 6538-P is suitable) in culture medium Cm4. Typical microbial growth must be observable after 24 hours of incubation at 30–32 °C.

Incubation

Incubate for 7 days the vessels containing fluid mercaptoacetate (thioglycolate) media (culture media Cm4 and Cm6) at 30–32 °C and the vessels containing soybean-casein digest media (media Cm5 and Cm7) at 22–25 °C. In the direct test procedure, gently agitate the vessels at least once a day or until complete dissolution occurs.

If other culture media are used, the incubation temperature and the period of incubation may have to be appropriately modified.

Examine inoculated culture vessels at regular intervals and on the last day of incubation for evidence of microbial growth. If such growth is observed it should be confirmed by microscopic examination. It is desirable that the incubation apparatus be equipped with a continuous temperature-recording device.

Interpretation of test results

If no evidence of growth is found in any of the culture vessels, except in the positive growth control, the material meets the requirements of the test. If, however, evidence of growth is found, a repeat test may be performed. If no evidence of growth is then found in any of the culture vessels, except in the positive growth control, the material meets the requirement of the test. The material fails to pass the test if growth occurs in the repeat test.

The distinction between failure of the product to pass the test and a possible invalidity of the test procedure requires the competent judgement of an expert.

3.3 Microbial purity of pharmaceutical preparations

Introduction

The following text is provided to give information and guidance, and is not regarded as an analytical requirement.

Microbial contamination of a product may lead not only to spoilage of the product, with the associated physical and chemical changes, but also to risk of infection for the user. Therefore, oral and topical pharmaceutical products (capsules, tablets, suspensions, creams, patches, etc.), which are not required to be sterile, should be subject to controls for microbial contamination.

Quality assurance and manufacturing controls should be such that organisms capable of proliferation and contamination of the product are within acceptable limits. The microbial limits and batch testing regimes set for the various categories of products should reflect the types of contamination most

likely to be introduced during manufacture. The intended use of the product also needs to be considered.

Microbial limits cannot be formulated to cover every possibility of contamination that may occur. In assessing the results of microbiological testing, the number and types of organisms present should be considered in the context of the proposed use of the product.

Thus, in the manufacture, packaging, storage, and distribution of pharmaceuticals, suitable measures must be taken to ensure their microbial quality.

Limits for medicinal plant materials and methods for detection and estimation of microorganisms are not included in this text. For such preparations reference should be made to *Quality control methods for medicinal plant materials* (WHO, 1998[1]): "Determination of microorganisms".

General requirements

Category	Dosage forms and materials	Recommended criteria
1	Preparations for injection Preparations for ophthalmic use	Sterility (see individual monographs)
2	Preparations for topical use Preparations for the respiratory tract (except those required to be sterile)	Total viable aerobic count not more than 10^2 bacteria and fungi per 1 g or 1 ml Not more than 10^1 enterobacteria and certain other Gram-negative bacteria per 1 g or 1 ml Absence of *Pseudomonas aeruginosa* in 1 g or 1 ml Absence of *Staphylococcus aureus* in 1 g or 1 ml
3	Preparations for oral administration Preparations for rectal administration	Total viable aerobic count not more than 10^3 aerobic bacteria per 1 g or 1 ml Not more than 10^2 fungi per 1 g or 1 ml Absence of *Escherichia coli* in 1 g or 1 ml
4	Preparations for oral administration containing materials of natural origin	Total viable aerobic count not more than 10^4 raw aerobic bacteria per 1 g or 1 ml Not more than 10^2 enterobacteria and certain other Gram-negative bacteria per 1 g or 1 ml Not more than 10^2 fungi per 1 g or 1 ml Absence of *Salmonella* in 10 g or10 ml Absence of *Escherichia coli* in 1 g or 1 ml Absence of *Staphylococcus aureus* in 1 g or 1 ml aerobic bacteria per 1 g or 1 ml

[1] This text is currently under revision.

3.4 Test for bacterial endotoxins

Introduction

The method for the detection of Gram-negative bacterial endotoxins is based on the gelation of a lysate of amoebocytes (limulus amoebocyte lysate, LAL) from the horseshoe crab, *Limulus polyphemus* or *Limulus tachypleus*. The addition of a solution containing endotoxins to a solution of the lysate produces turbidity, precipitation, or gelation of the mixture.

The rate of reaction depends on the concentration of endotoxin, the pH, and the temperature. The reaction requires the presence of certain divalent cations, an enzyme system, and protein capable of clotting, which are provided by the lysate.

The bacterial endotoxin test (BET) is carried out in a manner that avoids microbial contamination.

Before carrying out the test on the preparation to be examined, it is necessary to verify:

— that the equipment used does not adsorb endotoxins;
— the sensitivity of the lysate;
— the absence of interfering factors.

All equipment used must be free of endotoxins.

Recommended procedure

- Unless otherwise prescribed, the solutions and dilutions used in the test are prepared using water BET.

Pre-test. Adjust the solution to be examined to pH 6.5–7.5 using hydrochloric acid (0.1 mol/l) BET, sodium hydroxide (0.1 mol/l) BET, or a suitable buffer, if necessary. To each of the requisite number of chosen receptacles (for example, slides or tubes) add a volume of the lysate and maintain the temperature at 37 ± 1 °C. Then add to each receptacle an equal volume of the solution to be examined, mixing immediately and gently with the lysate. Incubate the reaction mixture without vibration, avoiding loss of water by evaporation, for a set period of time that has been determined under experimental conditions (usually 20–60 minutes), and check the results.

A positive result is indicated by the formation of a firm gel and no disintegration when the receptacle is gently inverted. If no such gel is formed the result is negative.

Validation. A cross-validation will need to be performed if the lysate used is different to the ones described in the "Introduction".

Sensitivity of the lysate. Prepare not fewer than four replicate series of two-fold dilutions of endotoxin RS to give concentrations of 2λ, 1λ, 0.5λ, and 0.25λ, where λ is the stated sensitivity of the lysate used. The final dilution in each series must at the minimum give a negative result. Examine the dilutions and a negative control solution consisting of water BET. Calculate the average of the logarithms of the lowest concentration of endotoxin in each series of dilu-

tions giving a positive result. The antilogarithm of this average gives the estimated lysate sensitivity. If the latter does not differ by more than a factor of 2 from the stated sensitivity, the sensitivity is confirmed and is used for all tests performed using the same lysate.

Interfering factors. Prepare as described under "Sensitivity of the lysate", but use untreated specimens of the preparation to be examined in which no endotoxins are detectable to prepare the dilutions of endotoxin RS. Use these specimens at the maximum valid dilution (MVD) calculated from the expression:

$$\text{maximum valid dilution} = \frac{\text{endotoxin limit concentration}}{\text{sensitivity of the lysate}}$$

both values being expressed in International Units (IU) of endotoxin per millilitre.

When the endotoxin limit concentration is specified in individual monographs in terms of IU of endotoxin per mg or per IU of product, multiply the endotoxin limit by the concentration of the product in the solution tested (in mg or IU of the product per ml of solution) to obtain the endotoxin limit concentration in IU of endotoxin per ml of solution tested. Where relevant, the multiplication applies to a reconstituted solution of the product as stated on the label.

The preparation to be examined may need to be treated if it contains interfering factors and acts as an inhibitor or an activator as determined under experimental conditions. Suitable treatments are dilution, filtration, neutralization, dialysis, or addition of substances that displace adsorbed endotoxins. The sensitivity of the lysate in the presence of the preparation to be examined should not differ by more than a factor of 2 from the sensitivity of the lysate alone. More sensitive lysates permit a greater dilution of the preparation to be examined and may contribute to the elimination of interference.

Interfering factors passing through a filter with a nominal separation limit corresponding to a relative molecular mass of 10 000 to 20 000 may be separated adequately by ultrafiltration. Asymmetric membrane filters of cellulose triacetate may be used. The presence of components causing false positive results must be determined. The material containing the endotoxins that is retained on the filter is rinsed with water BET or a suitable buffer, and the endotoxins are recovered in the water BET or the buffer. The test volume and the final volume used for the recovery of the endotoxins are determined for each preparation to be examined.

To establish if interfering factors have been eliminated without removing endotoxins, repeat the test using the preparation to be examined, add endotoxin RS, and submit it to the chosen treatment.

Preparation to be examined. Prepare in duplicate as described under "Pre-test", using the maximum valid dilution of the preparation to be examined, this having been treated if necessary for the elimination of interfering factors. At the same time examine a negative control consisting of water BET and two positive controls, both of which contain endotoxin RS at a concentration corresponding to twice the stated sensitivity of the lysate and one of which con-

tains the preparation to be examined at the same concentration as in the test (if necessary treated for the elimination of interfering factors after the addition of the endotoxin standard).

The test is valid if the negative and both positive controls give the appropriate result. The endotoxin limit concentrations of the preparations are given in the individual monograph. The product conforms if it complies with the endotoxin limit concentration. However, compliance with this requirement can only be demonstrated by showing that the endotoxin concentration of the product is less than the endotoxin limit concentration.

The preparation to be examined does not comply with the test if a positive result is found for both test mixtures. If a positive result is found for one test mixture and a negative result for the other, repeat the test.

The preparation to be examined complies with the test if a negative result is found for both test mixtures.

3.5 Test for pyrogens

The pyrogen test is designed to limit the risk of a febrile reaction following parenteral administration of drugs. It is intended to be used for liquid products that can be tolerated by the test rabbit in a dose of 10 ml per kg, injected intravenously, generally within a period of not more than 4 minutes. For products that require preliminary preparation or are subject to special conditions of administration, additional directions given in the monograph should be followed.

Test animal

Use healthy, adult rabbits, preferably of the same variety. House the animals individually in an area of uniform temperature (±2 °C), possibly with uniform humidity, and free from disturbances likely to excite them. The animals are given *ad libitum* water and food, commonly used for laboratory animals. One to 3 days before using an animal that has not previously been used for a pyrogen test, condition it by conducting a training exercise as described under the recommended procedure, omitting the injection.

Do not use animals for pyrogen tests more frequently than once every 48 hours. After a pyrogen test in the course of which a rabbit's temperature has risen by 0.5 °C or more, or after a rabbit has been given a test substance that was adjudged pyrogenic, at least 2 weeks must be allowed to elapse before the animal is used again.

Temperature recording

Use an accurate thermometer graduated in 0.1 °C that has been tested to determine the time necessary to reach the maximum reading, or any other temperature-recording device of equal sensitivity. Insert the temperature-sensing device into the rectum of the test animal to a depth of about 6 cm. If the temperature-sensing device is to remain inserted throughout the sensing period,

restrain the rabbit with a lightly-fitting neck stock that allows it to assume a natural resting posture. When a thermometer is used, allow sufficient time for it to reach a maximum temperature, as previously determined, before taking the reading.

Recommended procedure

Perform the test in the area where the animals are housed or under similar environmental conditions. For 2 hours before the test and during the test, withhold all food from the animals being used. Access to water may be allowed. The animals should be placed under the conditions of the test at least 1 hour before the injection.

Prior to the test, 40 minutes before the injection of the test material, determine the temperature of each animal by taking 2 measurements at an interval of 30 minutes. The mean of the 2 temperatures serves as the "control temperature" of the animal. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated.

In any one test, use only those animals the control temperatures of which do not deviate by more than 1.0 °C from each other. Those animals for which the 2 temperatures used to determine the control temperature have deviated by more than ±0.2 °C from the mean should not be used in the test, nor should any animal with a control temperature below 38.0 °C or above 39.8 °C.

Render the syringes, needles, and glassware free from pyrogens by heating at 250 °C for not less than 30 minutes or by any other suitable method. Warm the solution to be tested to approximately 38 °C.

Inject into a marginal vein of the ear of each of 3 rabbits 10 ml of the solution per kg of body weight or the amount specified in the monograph. The injection should last not longer than 4 minutes, unless otherwise specified in the monograph.

When the injection has been completed, record the temperature of the animal during a period of 3 hours, taking the measurements continuously or every 30 minutes. The maximum temperature recorded for each rabbit is considered to be its response; if the temperature readings taken after the injection are all below the control temperature, the response is treated as a zero temperature rise.

If no rabbit shows an individual rise in temperature of 0.6 °C or more above its respective control temperature, and if the sum of the 3 temperature rises does not exceed 1.4 °C, the tested material meets the requirements for the absence of pyrogens. If 1 or 2 rabbits show a temperature rise of 0.6 °C or more, or if the sum of the temperature rises exceeds 1.4 °C, continue the test using 5 other rabbits. If not more than 3 of the 8 rabbits show individual rises in temperature of 0.6 °C or more, and if the sum of the 8 temperature rises does not exceed 3.7 °C, the tested material meets the requirements for the absence of pyrogens.

3.6 Test for histamine-like substances (vasodepressor substances)

The test for vasodepressor substances is carried out in cats by comparing the depression of arterial pressure caused by the test solution with that obtained after administration of a solution of histamine.

Recommended procedures

Use healthy, adult cats, either males or non-pregnant females.

Determine the weight of the animal and place under general anaesthesia by injection of chloralose R or a suitable barbiturate that allows the maintenance of uniform blood pressure. Protect the animal from loss of body heat and maintain it so that the rectal temperature remains within physiological limits. Introduce a tube into the trachea. Surgically expose the common carotid artery and by blunt dissection separate it completely from all surrounding structures, including the vagus nerve. Insert a cannula filled with heparinized saline TS into the artery and connect it to a mercury manometer or another suitable device arranged for making a continuous record of blood pressure. Surgically expose the jugular or the femoral vein and insert into it another cannula filled with heparinized saline TS through which can be injected solutions of histamine and of the test substance.

Determine the sensitivity of the animal to histamine in the following way: Start the recording kymograph or a similar recording device and inspect the tracings for amplitude of excursion and relative stability of blood pressure. Inject into the jugular or femoral vein histamine TS, in doses of 0.05 µg (dose A), 0.1 µg (dose B) – repeated at least 3 times – and 0.15 µg (dose C) of histamine base per kg of animal weight. Administer the second and subsequent injections not less than 1 minute after the blood pressure has returned to the level recorded immediately before the previous injection. Repeat this series of injections until, disregarding the first series of readings, a relatively uniform decrease in blood pressure is obtained after doses B of histamine. The animal should be used for the test only if the decrease after doses B is not less than 2.7 kPa (20 mm Hg) and, moreover, if dose A causes smaller responses than doses B whereas dose C gives greater responses than doses B.

Prepare the test solution as described in the monograph. During the course of the test, take care to maintain a uniform rate of injection for both the test solution and the standard solution. If the jugular vein is used, care should also be taken that the injection of test solution and histamine standard are given in equal volumes to avoid volume effects on blood pressure. When a common cannula is used for both the standard and test solutions, each injection of the standard and test solution should be immediately followed by an injection of approximately 2.0 ml of saline TS to flush any residues from the tubing.

Inject a dose B of the standard solution followed by an injection of the specified amount of the test solution and then another dose B of the standard solution. The second and third injections are given not less than 1 minute after the

blood pressure has returned to the level recorded immediately before the preceding injection. If the response to the test solution is greater than that given previously by dose A, repeat the series of injections twice and conclude the test by giving dose C of standard solution. If the response to dose C is not greater than that to dose B, the test is invalid.

The animal may be used in the test as long as it remains reasonably stable and responsive to histamine and provided that (*a*) an injection of test substance did not cause a greater depressor response than that caused by dose C and (*b*) the response to dose C of the standard solution given after the administration of the test substance does not become lower than the mean response to doses of B previously injected.

The substance passes the test if the response or the mean of the responses after the injection of the amount specified in the monograph is smaller than the mean of the corresponding responses to dose B of the standard solution (0.1 µg of histamine base per kg of animal weight), and no one single dose of the test solution causes a greater depressor response than dose C of the standard solution (0.15 µg of histamine base per kg of animal weight).

3.7 Undue toxicity

The test is used to determine the absence of undue toxicity of antibiotics intended for parenteral administration.

Recommended procedure

Use healthy mice of a single strain that have not previously been used for any test. Select 5 mice, each weighing between 18 g and 22 g. Prepare the solution of the test substance as specified in the monograph. Inject a test dose of 0.5 ml intravenously into a tail vein at a uniform rate, the injection occupying 5 seconds. Keep the mice under observation for 48 hours after the injection. The product meets the requirements for freedom from undue toxicity if no animal dies within 48 hours.

If 1 or 2 mice die within the observation period, repeat the procedure once, using respectively 5 or 15 mice, healthy and not previously used for any test, each weighing between 19.5 g and 20.5 g. The product under test meets the requirements for freedom from undue toxicity if no animal dies in the repeat test within the observation period (48 hours).

4. Methods for materials of plant origin

4.1 Determination of ash and acid-insoluble ash

Recommended procedures

Determination of ash

Place about 3 g of the ground material, accurately weighed, or the quantity specified in the monograph, in a suitable tared dish (for example, of silica or platinum), previously ignited, cooled and weighed. Incinerate the material by gradually increasing the heat, not exceeding 450 °C, until free from carbon; cool, and weigh. If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter-paper, incinerate the residue and filter-paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 °C. Calculate the content in mg of ash per g of air-dried material.

Determination of acid-insoluble ash

Boil the ash for 5 minutes with 25 ml of hydrochloric acid (~70 g/l) TS; collect the insoluble matter in a sintered crucible, or on an ashless filter-paper, wash with hot water, and ignite at about 500 °C to constant weight. Calculate the content in mg of acid-insoluble ash per g of air-dried material.

4.2 Determination of iodine value

The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance. The quantity of substance used in the determination should be such that at least 70% of the iodine added, as provided in the recommended procedure, is not absorbed. Unless otherwise specified in the monograph, the quantity of the substance indicated in the following table should be used for the determination, depending on the expected iodine value:

Iodine value	Quantity of substance in g
less than 20	1.0
20–60	0.5–0.25
60–100	0.25–0.15
more than 100	0.15–0.10

Recommended procedure

Place a quantity of the test substance, accurately weighed, as specified in the monograph, in a dry 300-ml to 500-ml stoppered flask, add 15 ml of carbon tetrachloride R and dissolve. Add 25 ml of iodine bromide TS, insert the stopper,

previously moistened with potassium iodide (80 g/l) TS, shake the flask gently, and keep in the dark for 30 minutes, unless otherwise specified in the monograph. Add 20 ml of potassium iodide (80 g/l) TS and 150 ml of water, and, whilst shaking the contents of the flask, titrate with sodium thiosulfate (0.1 mol/l) VS, adding starch TS as indicator towards the end of the titration. Note the number of ml required (*a*). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of sodium thiosulfate (0.1 mol/l) VS required (*b*). Calculate the iodine value from the following formula:

$$\text{Iodine value} = \frac{(b - a) \times 0.01269 \times 100}{\text{weight(ing) of substance}}$$

4.3 Determination of peroxides in fixed oils

Recommended procedure

Dissolve the quantity of test substance as specified in the monograph, usually about 3 g, accurately weighed, in 15 ml of chloroform R and 30 ml of glacial acetic acid R in a 250-ml glass-stoppered flask. Add 1 ml of a freshly prepared solution of 1.3 g of potassium iodide R in 1 ml of water, stopper the flask, mix by gentle swirling and set aside in the dark for 3 minutes. Add 100 ml of water, shake, and titrate with sodium thiosulfate (0.01 mol/l) VS, using starch TS as indicator. Repeat the operation without the substance being tested and calculate the difference between the titrations, the limit value being specified in the monograph.

4.4 Determination of saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of the substance.

In the procedure described, a 50-ml burette should preferably be used for titration, as in the blank titration the volume of hydrochloric acid (0.5 mol/l) VS used is exactly 35.5 ml when the concentration of ethanolic potassium hydroxide is exactly 40 g/l.

Recommended procedure

Place about 2 g of the test substance, accurately weighed, or the quantity specified in the monograph, in a flask with a capacity of about 200 ml, add 25 ml of potassium hydroxide/ethanol, TS1 attach a reflux condenser, and heat in a boiling water-bath for 30 minutes, or the time specified in the monograph, frequently rotating the contents of the flask; immediately add 1 ml of phenolphthalein/ethanol TS and titrate the excess of alkali with hydrochloric acid (0.5 mol/l) VS. Note the number of ml of hydrochloric acid (0.5 mol/l) VS required to titrate the sample (*a*). Repeat the operation without the substance being tested

and note the number of ml of hydrochloric acid (0.5 mol/l) VS required for neutralization (b). Calculate the saponification value from the following formula:

$$\text{Saponification value} = \frac{(b-a) \times 0.02805 \times 1000}{\text{weight(ing) of substance}}$$

4.5 Determination of unsaponifiable matter

The term "unsaponifiable matter" refers to those substances present in oils or fats that are not saponified by alkali hydroxides and are extractable into ether.

Recommended procedure

Place a quantity of the test substance, accurately weighed, as specified in the monograph, in a flask provided with a reflux condenser and boil in a water-bath for 1 hour with 25 ml of potassium hydroxide/ethanol (0.5 mol/l) VS, with frequent swirling of contents. Wash the contents of the flask into a separator by means of 50 ml of water and, while the liquid is still slightly warm, extract by shaking vigorously with 3 successive quantities, each of 50 ml, of ether R, washing out the flask with the first quantity of ether R. (CAUTION: Ether should be free of peroxides.) Take care to release frequently and carefully the pressure that may build up inside the separator. Combine the ethereal solutions in another separator containing 20 ml of water. (If the ethereal solutions contain solid suspended matter, filter them into the separator through a fat-free filter-paper and wash the filter-paper with ether R.) Gently rotate the separator for a few minutes without violent shaking, allow the liquids to separate, and run off the aqueous layer. Wash the ethereal solution by shaking vigorously with 2 successive quantities, each of 20 ml, of water; then treat 3 times with 20 ml of potassium hydroxide (0.5 mol/l) VS (NOTE: aqueous reagent), shaking vigorously on each occasion and washing with 20 ml of water after each treatment. Finally wash with successive quantities, each of 20 ml, of water until the aqueous layer is no longer alkaline to phenolphthalein/ethanol TS. Transfer the ethereal extract to a weighed flask, washing out the separator with ether R; distil off the ether with the necessary precautions and add 3 ml of acetone R.

By the aid of a gentle current of air remove the solvent completely from the flask, which is preferably held obliquely and rotated, almost entirely immersed, in a water-bath at about 60 °C. Dry to constant weight at a temperature not above 80 °C and dissolve the contents of the flask in 10 ml of ethanol (~750 g/l) TS, previously neutralized to phenolphthalein/ethanol TS. Titrate with carbonate-free sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. If the amount of carbonate-free sodium hydroxide (0.1 mol/l) required does not exceed 0.2 ml, the amount weighed is to be taken as the unsaponifiable matter. Calculate the unsaponifiable matter as a percentage of the oil or fat. If the amount of carbonate-free sodium hydroxide (0.1 mol/l) VS required exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

4.6 Determination of acid value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance.

Recommended procedure

Accurately weigh about 10 g of the substance, or the quantity specified in the monograph, into a 250-ml flask, and add 50 ml of a mixture of equal volumes of ethanol (~750 g/l) TS and ether R, which has been neutralized with potassium hydroxide (0.1 mol/l) VS after the addition of 1 ml of phenolphthalein/ethanol TS. Heat, if necessary, until the substance has completely dissolved, cool; titrate with potassium hydroxide (0.1 mol/l) VS, constantly shaking the contents of the flask until a pink colour, which persists for 15 seconds, is obtained. Note the number of ml required (*a*). Calculate the acid value from the following formula:

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{\text{weight(ing) of substance}}$$

4.7 Determination of hydroxyl value

The hydroxyl value of a substance is the amount, in milligrams, of potassium hydroxide required to neutralize any acid when combined by acylation in 1 g of the substance under examination.

Recommended procedures

Method A

To the quantity of the substance being examined (as specified in the individual monograph) add 12 g of stearic anhydride R and 10 ml of xylene R and heat gently under a reflux condenser for 30 minutes. Allow to cool, add a mixture of 40 ml of pyridine R and 4 ml of water, and heat again under a reflux condenser for 30 minutes. Titrate the hot solution with carbonate-free sodium hydroxide (1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure, omitting the substance under examination.

The hydroxyl value is calculated from the expression 56.10 *v/m*, where *v* is the difference, in ml, between the two titrations and *m* is the quantity, in g, of the substance taken.

Method B

Unless otherwise indicated in the individual monograph, weigh accurately the quantity of the substance to be examined shown in the table under 4.7 Determination of hydroxyl value, place it in a 150-ml acetylation flask fitted with an air condenser and add the corresponding volume of pyridine/acetic anhydride TS.

Presumed hydroxyl value	Quantity of substance (g)	Volume of pyridine/acetic anhydride TS (ml)
10–100	2.0	5.0
100–150	1.5	5.0
150–200	1.0	5.0
200–250	0.75	5.0
250–300	0.60 or 1.20	5.0 or 10.0
300–350	1.0	10.0
350–700	0.75	15.0
700–950	0.5	15.0

Heat the flask for 1 hour in a water-bath, maintaining the level of the water 2–3 cm above the level of the liquid in the flask. Remove the flask and condenser, allow to cool, and add 5 ml of water through the top of the condenser. If a cloudiness appears, add sufficient pyridine R to produce a clear liquid, noting the volume added. Shake the flask, place it in a water-bath for 10 minutes, remove, and allow to cool. Rinse the condenser and the walls of the flask with 5 ml of neutralized ethanol TS. Titrate with potassium hydroxide/ethanol (0.5 mol/l) VS, using 0.2 ml of phenolphthalein/ethanol TS as indicator. Repeat the procedure, omitting the substance under examination.

Calculate the hydroxyl value from the expression $(a + 28.05)\ v/m$, where v is the difference, in ml, between the two titrations, a is the acid value determined for the substance, and m is the quantity, in g, of the substance taken.

5. Pharmaceutical technical procedures

Introduction

In addition to general methods of analysis that are invoked within the general monographs for different types of dosage forms, for example, dissolution testing, this section contains other general texts of relevance during the development and manufacture of pharmaceutical dosage forms and to which reference is made within the monographs of the *International Pharmacopoeia*.

5.1 Uniformity of content for single-dose preparations

This test should be applied only where the declared quantity of active ingredient in tablets, capsules, or suppositories is 5% or less of the total formulation or, in the case of sugar-coated and enteric-coated tablets, where the test for 5.2 Uniformity of mass for single-dose preparations does not apply, or for certain powders for injections when specified in individual monographs.

Recommended procedure
Individually, determine the amount of active ingredient in each of 10 units using the analytical method specified in the individual monograph for 5.1 Uniformity of content for single-dose preparations or, where no specific method is stated, refer to the procedure for the "Assay" in the appropriate monograph.

Requirements for tablets and powders for injections
Each single unit should contain within +15% of the average amount of the active ingredient. However, if one individual unit deviates by more than ±15% but is within ±25% of the average amount of the active ingredient, examine a further 20 units drawn from the same original sample as the first 10 units. The preparation under test complies only if the amount of active ingredient found in no more than one out of 30 units deviates by more than ±15% of the average amount. None should deviate by more than ±25% of the average amount.

Requirements for capsules, oral powders and suppositories
Each single unit should contain within ±15% of the average amount of active ingredient. However, if up to three individual units deviate by more than ± 15% but are within ± 25% of the average amount of the active ingredient, examine a further 20 units drawn from the same original sample as the first 10 units. The preparation under test complies only if the amount of active ingredient found in no more than three out of 30 units deviates by more than ± 15% of the average amount. None should deviate by more than ± 25% of the average amount.

5.2 Uniformity of mass for single-dose preparations

Tablets

Uncoated tablets and film-coated tablets formulated to contain 5% or more of the active ingredient should comply with the following test.

Recommended procedure
Weigh 20 tablets and calculate the average mass. When weighed singly, the deviation of individual masses from the average mass should not exceed the limits given below.

Average mass of tablet	Deviation %	Number of tablets
less than 80 mg	±10.0	minimum 18
	±20.0	maximum 2
80 mg to 250 mg	±7.5	minimum 18
	±15.0	maximum 2
more than 250 mg	±5.0	minimum 18
	±10.0	maximum 2

If film-coated tablets fail this test it may be because of variability in the thickness (mass) of the coatings. In such a case, a test for 5.1 Uniformity of content for single-dose preparations should be applied; if the tablets meet the requirement of this test, they can be considered acceptable.

Capsules

Recommended procedure

Weigh 20 intact capsules individually, and calculate the average mass. The mass of each capsule should be within ±10% of the average mass. If all the capsules do not fall within these limits, weigh the 20 capsules again, taking care to preserve the identity of each capsule, and remove the contents as completely as possible. For soft gelatin capsules, wash the shell with ether or some other suitable solvent and allow it to stand until the odour of the solvent is no longer perceptible. Other means, such as a jet of compressed air, may be used to remove the contents.

Weigh the emptied shells individually and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the gross mass. Determine the average net content from the sum of the individual net masses. Then determine the difference between each individual net content and the average net content. Deviation of individual net mass from the average net mass should not exceed the limits given below.

Net mass of capsule contents	Deviation %	Number of capsules
less than 300 mg	±10.0	minimum 18
	±20.0	maximum 2
300 mg and over	±7.5	minimum 18
	±15.0	maximum 2

Oral powders

Weigh 20 units and calculate the average mass. The deviation of individual mass from the average mass should not exceed the limits given above under "Capsules."

Powders for injections

The test applies to powders for injections where the content is more than 40 mg. The recommended procedure is the same as described for capsules. The deviation of individual net mass from the average net mass should not exceed the limits given below.

Deviation %	Number of containers
±10	minimum 18
±20	maximum 2

For preparations with a content of less than 40 mg the test for 5.1 Uniformity of content for single-dose preparations applies instead.

Suppositories

Weigh 20 suppositories and calculate the average mass. When the suppositories are weighed singly, the deviation of individual mass from the average mass should not exceed the limits given below.

Deviation %	Number of suppositories
±5	minimum 18
±10	maximum 2

5.3 Disintegration test for tablets and capsules

This test determines whether tablets and capsules disintegrate within a prescribed time when placed in an immersion fluid under prescribed experimental conditions. Disintegration is defined as the state in which no residue of the tablet or capsule, except fragments of undissolved coating or capsule shell, remains on the screen of the test apparatus or, if any other residue remains, it consists of a soft mass having no palpably firm, unmoistened core.

Disintegration apparatus

The apparatus consists of a circular basket-rack assembly, a suitable vessel for the immersion fluid (such as a 1-litre beaker), a thermostatic arrangement for maintaining the fluid at the required temperature (normally 37 ± 2 °C), and a device for raising and lowering the basket-rack in the immersion fluid at a constant frequency of 28–32 cycles/min through a distance of 50–60 mm.

The basket-rack assembly consists of six open-ended cylindrical glass tubes and a rack for holding them in a vertical position. The tubes are 75–80 mm long, and have an inside diameter of about 21.5 mm and a wall about 2 mm thick. The tubes are held vertically by two superimposed plates, circular in shape and made of transparent plastic material, each about 90 mm in diameter and 6 mm thick, perforated by six holes of a diameter that allows the tubes to be inserted. The holes are equidistant from the centre of the plate and equally spaced one from another. A piece of woven gauze, made of stainless steel wire about 0.635 mm in diameter, with a mesh aperture of 2.0 mm is attached to the under-

side of the lower plate. The upper plastic plate is covered with a stainless steel plate, about 1 mm thick, of a diameter similar to that of the plastic plates. The steel plate is perforated by six holes about 22 mm in diameter, positioned to coincide with those of the upper plastic plate. It is placed over the tubes and consolidates the whole structure. The plates are held rigidly 75–80 mm apart by vertical stainless steel rods at the periphery. A metal rod is fixed to the centre of the upper plate. This enables the assembly to be attached to a suitable mechanical device so that it may be lowered and raised.

The volume of the fluid in the immersion vessel should be such that, at the highest point of the upward stroke, the wire mesh that forms the bottom of the basket remains at least 25 mm below the surface of the fluid. At the lowest point of the downward stroke, it should descend to not less than 25 mm from the bottom of the vessel. The time required for the upward stroke should be equal to the time required for the downward stroke, and the change in stroke direction should be a smooth transition rather than an abrupt reversal of motion.

Where a disc is prescribed in the monograph, the following configuration and dimensions apply: a cylindrical disc 20.7 ± 0.15 mm in diameter and 9.5 ± 0.15 mm thick, made of transparent plastic with a relative density of 1.18 to 1.20. Each disc is pierced by five holes 2 mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6 mm from the centre of the disc. On the lateral surface of the disc, four equally spaced grooves are cut so that on the upper surface of the disc they are 9.5 mm wide and 2.55 mm deep and, at the lower surface, 1.6 mm square.

Different designs of basket-rack assembly may be used, provided that the specifications for the glass tubes and the stainless steel wire gauze are maintained.

Recommended procedure (except for effervescent tablets)
Unless otherwise specified in the individual monograph, use water as the immersion fluid at a temperature of 37 ± 2 °C. Place one tablet or capsule in each of the six tubes and, if prescribed, add a disc to each tube. Operate the apparatus for the specified period of time, withdraw the assembly, and examine the state of the tablets or capsules. All six tablets or capsules should disintegrate to pass the test.

5.4 Disintegration test for suppositories

The disintegration test determines whether suppositories soften or disintegrate within a prescribed time when placed in an immersion fluid using the experimental conditions described below.

Disintegration is considered to be achieved when:

— dissolution is complete;
— the components of the suppositories have separated, e.g. melted fatty substances have collected on the surface of the liquid, insoluble powders

have fallen to the bottom, and soluble components have dissolved or are distributed in one or more of the ways described in Methods 1 and 2;
— there is softening of the test sample, usually accompanied by an appreciable change of shape without complete separation of the components. The softening process is such that a solid core no longer exists when pressure is applied with a glass rod; or
— rupture of the gelatin shell or rectal capsule occurs resulting in release of the contents.

Method 1 (for water-soluble, hydrodispersible and fat-based suppositories):

This test measures the time elapsed for a suppository placed in water to disintegrate.

Apparatus

The apparatus (Fig. 1) consists of a 60-mm long cylinder of glass or transparent plastic and a metal device consisting of two perforated stainless steel discs, held about 30 mm apart. These discs each have 39 holes, 4 mm in diameter, which are evenly spaced in a concentric pattern. The diameter of the discs is marginally inferior to that of the interior of the cylinder. Once inserted into the cylinder, the metal device is attached to the rim of the cylinder by means of three spring clips. The test is carried out using three such apparatuses, each containing a single test sample. Each apparatus is placed in a beaker with a minimum capacity of 4 litres filled with water unless otherwise prescribed. The beaker is fitted with a slow stirrer and a support that holds the apparatus vertically 90 mm below the surface of the water so that it can be inverted without emerging from the water.

Recommended procedure

Unless otherwise described in the individual monograph, use water maintained at a temperature of 36–37 °C as the immersion fluid. The test requires three suppositories and the procedure is applied to each of the suppositories.

Place the sample on the lower disc of the metal device and then insert it into the cylinder. Place the apparatus into the beaker and invert it every 10 minutes without removing it from the liquid. Repeat the operation with the remaining two suppositories. Record the time required for the disintegration of the suppositories.

Unless otherwise stated in the individual monograph, for each of the three suppositories, examine the state of the sample after 30 minutes for fat-based suppositories and rectal capsules, and after 60 minutes for water-soluble suppositories.

Method 2 (alternative for fat-based suppositories):

This test measures the time elapsed for a suppository placed in water to soften to the extent that it no longer offers resistance when a defined weight is applied.

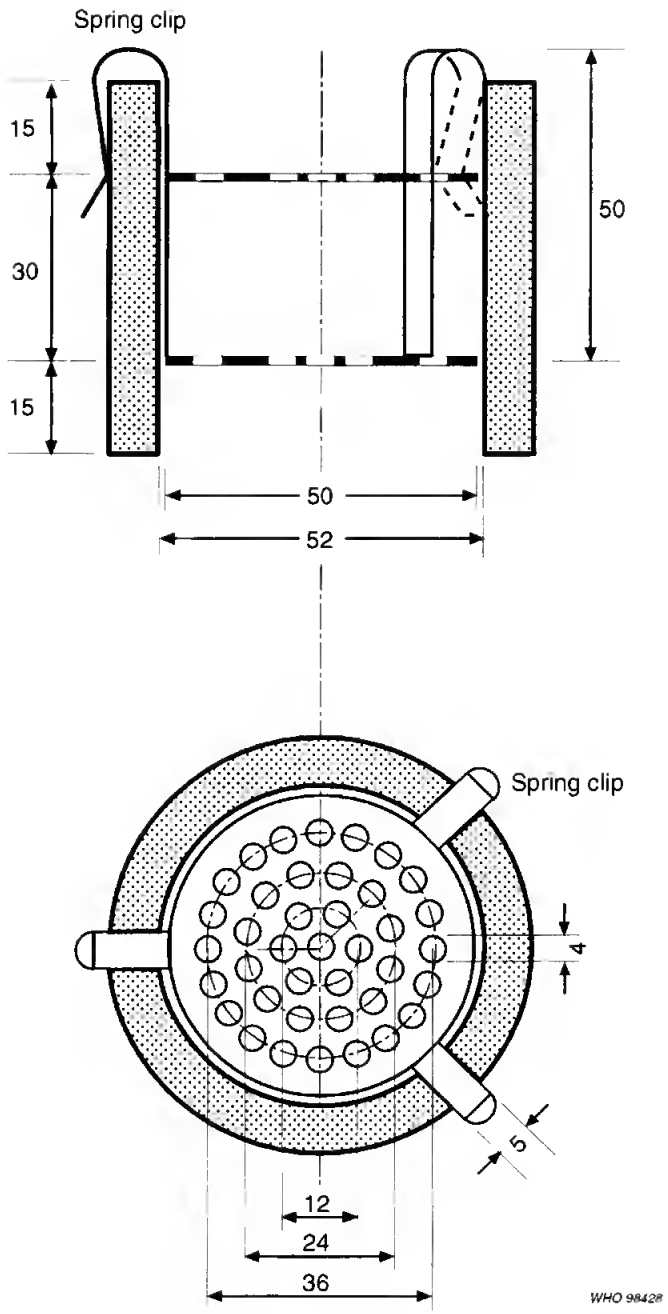


Figure 1. Apparatus for water-soluble, hydrodispersible, and fat-based suppositories
A. Horizontal view
B. Vertical view
Measurements in mm.

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Apparatus

The apparatus (Fig. 2) consists of a flat-bottomed glass tube about 140 mm long with an internal diameter of 15.5 mm and a two-part rod. The tube is closed with a removable plastic cover that has an opening 5.2 mm in diameter. The rod has two parts: both made of plastic, or the lower part made of plastic and the upper of metal. The rod is 5 mm in diameter and widens at the lower end to a diameter of 12 mm. To the bottom of the lower end is fixed a metal needle 2 mm long and 1 mm in diameter. The upper part of the rod has an adjustable sliding ring and a weighted disc is attached to the top. The two parts are held tightly together for the manual version or separated for the automatic version. The weight of the entire rod should be 30 g ± 0.1 g.

Recommended procedure

Unless otherwise described in the individual monograph, use water maintained at a temperature of 36–37 °C as the immersion fluid. The test requires three suppositories and the procedure is applied to each of the suppositories.

Place the glass tube containing 10 ml of water in the water-bath and equilibrate at 36.5 ± 0.5 °C. Fix the glass tubes vertically and immerse to a depth of at least 7 cm below the surface but without touching the bottom of the water-bath. Introduce a suppository, tip first, into the tube followed by the rod with the free gliding plastic cover into the glass tube until the metal needle touches the flat end of the suppository. Put the cover on the tube. Note the time which elapses until the rod sinks down to the bottom of the glass tube and the mark ring reaches the upper level of the plastic cover.

Each of the three suppositories should melt within 30 minutes, unless otherwise stated in the individual monograph.

5.5 Dissolution test for solid oral dosage forms

This test determines the amount of active ingredient(s) released from a solid oral dosage form, such as a tablet or a capsule, using a known volume of dissolution medium within a predetermined length of time. This test method may not be applicable to certain oral dosage forms.

Apparatus

All parts of the apparatus, including any metal that may come into contact with the sample to be tested or the dissolution medium, should be made from a chemically inert material and should not adsorb, react or interfere with the preparation or the dissolution medium.

The dissolution assembly should be constructed in such a way that any vibration is reduced to a minimum.

Use an apparatus that allows full visibility of all operations.

The apparatus "Paddle" (Fig. 3) consists of a cylindrical vessel of suitable glass or other suitable transparent material with a hemispherical bottom and a nominal capacity of 1000 ml. The vessel is covered to prevent evaporation of

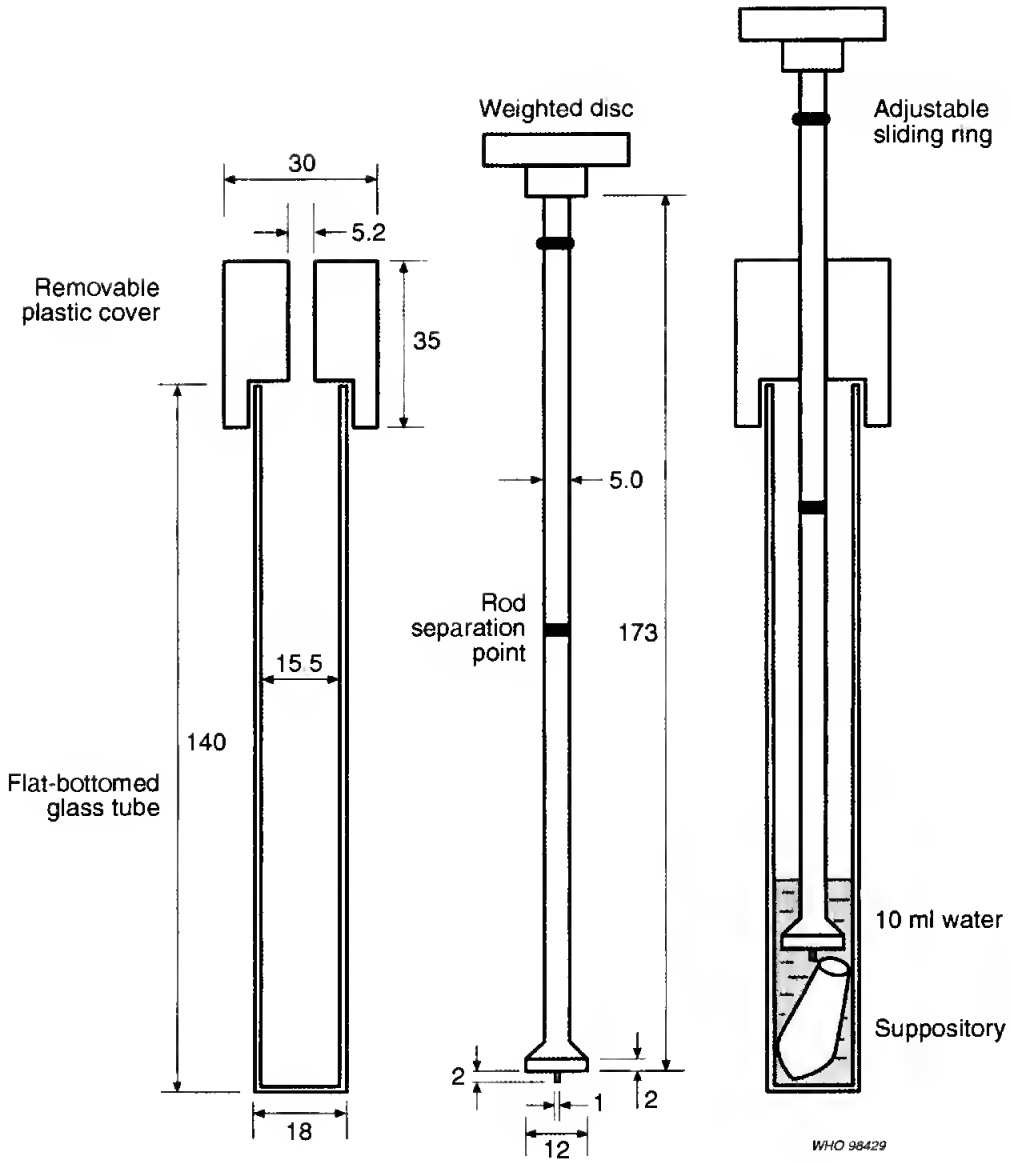

Figure 2. Alternative apparatus for fat-based suppositories
Measurements in mm.
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the medium with a cover that has a central hole to accommodate the shaft of the stirrer and other holes for the thermometer and for devices for withdrawal of liquid. The stirrer consists of a vertical shaft with a blade at the lower end. The blade is constructed around the shaft so that it is flush with the bottom of the shaft. When placed inside the vessel, the shaft's axis is within 2 mm of the axis of the vessel and the bottom of the blade is 25 ± 2 mm from the inner bottom of the vessel. The upper part of the shaft is connected to a motor pro-

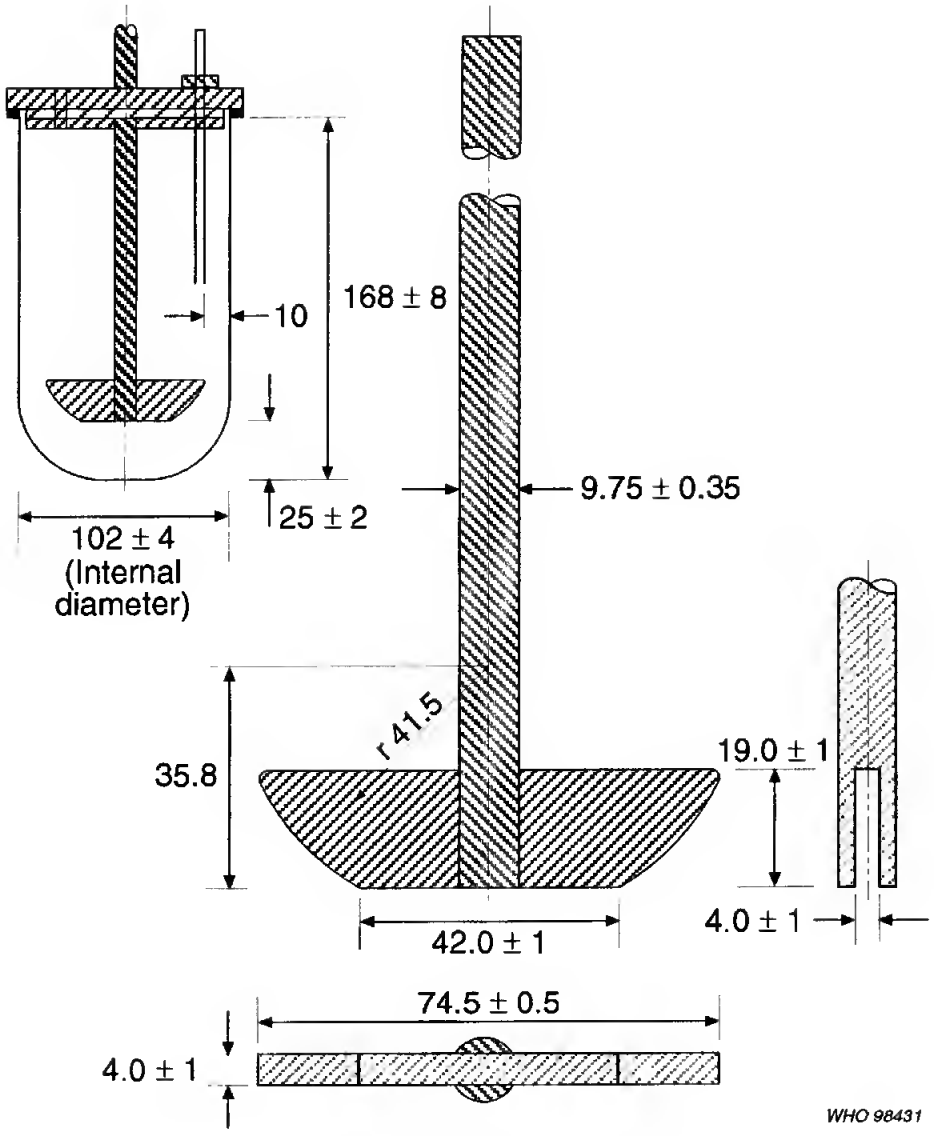

Figure 3. Paddle

Measurements in mm.

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vided with a speed regulator so that smooth rotation of the stirrer can be maintained without any significant wobble. The apparatus is placed in a water-bath that maintains the dissolution medium in the vessel at 37 ± 0.5 °C.

The apparatus "Basket" (Fig. 4) consists of the same apparatus as described for "Paddle", except that the paddle stirrer is replaced by a basket stirrer. The basket consists of two parts. The top part, with a vent, is attached to the shaft. It is fitted with three spring clips, or other suitable attachments, that allow

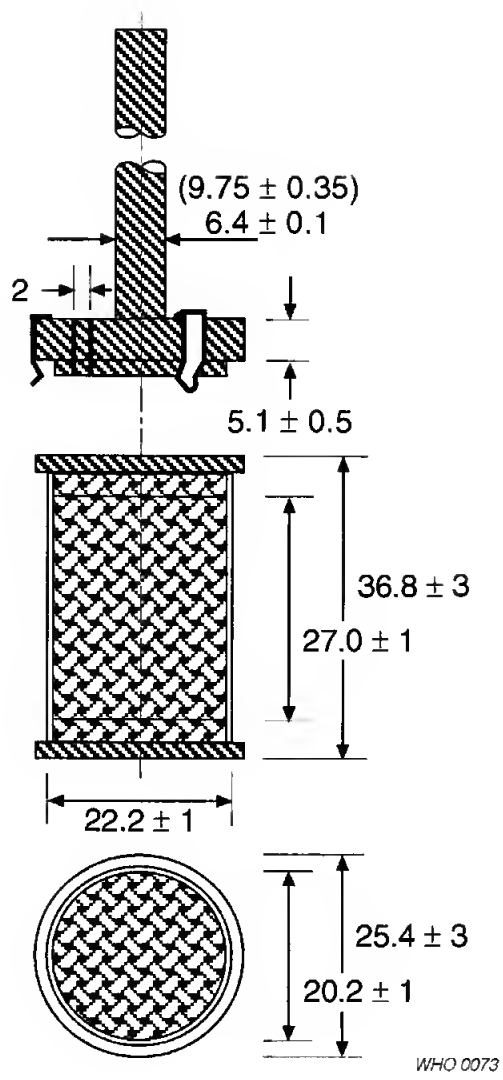

Figure 4. Basket
Measurements in mm.
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removal of the lower part so that the preparation being examined can be placed in the basket. These three spring clips firmly hold the lower part of the basket concentric with the axis of the vessel during rotation. The lower detachable part of the basket is made of welded-seam cloth, with a wire thickness of 0.254 mm diameter and with 0.381 mm square openings, formed into a cylinder with a narrow rim of sheet metal around the top and the bottom. If the basket is to be used with acidic media, it may be plated with a 2.5-μm layer of gold. When placed inside the vessel, the distance between the inner bottom of the vessel and the basket is 25 ± 2 mm.

Test conditions

The following specifications are given in the individual monographs:

— the apparatus to be used;
— the composition and volume of the dissolution medium;
— the rotation speed of the paddle or basket;
— the preparation of the sample and reference solutions;
— the time, the method, and the amount for sampling of the test solution or the conditions for continuous monitoring;
— the method of analysis; and
— the limits of the quantity or quantities of active ingredient(s) required to dissolve within a prescribed time.

Dissolution media

If a buffer is added to the dissolution medium, adjust its pH to within ±0.05 units of the prescribed value. Prior to testing, if necessary, remove any dissolved gases that could cause the formation of bubbles.

- **Dissolution buffer pH 1.3, TS**

Dissolve 2 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 1.3 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Dissolution buffer pH 2.5, TS**

Dissolve 2 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 2.5 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Dissolution buffer pH 3.5, TS**

Dissolve 7.507 g of glycine R and 5.844 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 3.5 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Dissolution buffer pH 4.5, TS**

Dissolve 6.8 g of potassium dihydrogen phosphate R in 900 ml of deionized water, adjust the pH to 4.5 either with hydrochloric acid (~70 g/l) TS or sodium hydroxide (~80 g/l) TS, and dilute to 1000 ml with water.

- **Dissolution buffer pH 6.8, TS**

Dissolve 5.53 g of disodium hydrogen phosphate R in 800 ml of deionized water, adjust the pH to 6.8 with sodium hydroxide (~80 g/l) TS, and dilute to 1000 ml with water.

- **Dissolution buffer pH 7.2, TS**

Dissolve 9.075 g of potassium dihydrogen phosphate R in deionized water to produce 1000 ml (solution A). Dissolve 11.87 g of disodium hydrogen phosphate R in sufficient water to produce 1000 ml (solution B). Mix 300 ml of solution A with 700 ml of solution B.

- **Gastric fluid, simulated, TS**

Dissolve 2.0 g of sodium chloride R and 3.2 g of pepsin R in 7.0 ml of hydrochloric acid (~420 g/l) TS and sufficient water to produce 1000 ml. This test solution has a pH of about 1.2.

- **Intestinal fluid, simulated, TS**

Dissolve 6.8 g of potassium dihydrogen phosphate R in 250 ml of water, mix, and add 190 ml of sodium hydroxide (0.2 mol/l) VS and 400 ml of water. Add 10.0 g of pancreatin R, mix, and adjust the resulting solution with sodium hydroxide (0.2 mol/l) VS to a pH of 7.5 ± 0.1. Dilute with sufficient water to produce 1000 ml.

Acceptance criteria

The requirements are met if the quantities of active ingredient(s) dissolved from the dosage forms tested conform to the following table, unless otherwise specified in the individual monograph.

Stage	Samples tested	Acceptance criteria
S_1	6	Each unit is not less than $Q + 5\%$
S_2	6	Average of 12 units ($S_1 + S_2$) is equal to or greater than Q, and no unit is less than Q-15%
S_3	12	Average of 24 units ($S_1 + S_2 + S_3$) is equal to or greater than Q; not more than 2 units are less than Q-15%; no unit is less than Q-25%

Continue testing through the three stages unless the results conform at either S_1 or S_2. The quantity, Q, is the released labelled content of active ingredient as a percentage as specified in the individual monograph; both the 5% and 15% values in the acceptance table are percentages of the labelled content so that these values and Q are in the same terms.

Recommended procedure

(See also guidance under *Details of the procedure* below.)

Ensure that the equipment has been calibrated within the past 6–12 months. Place the volume of dissolution medium, as stipulated in the individual monograph, in the vessel; assemble the apparatus and place it in the water-bath; allow the temperature of the dissolution medium to reach 37 ± 0.5 °C and remove the thermometer.

When apparatus "Paddle" is used, allow either one tablet or one capsule of the preparation to be tested to sink to the bottom of the vessel before starting the rotation of the blade, taking care that no air bubbles are present on the surface of the dosage form. In order to stop the dosage form from floating, anchor it to the bottom of the vessel using a suitable device such as a wire or glass helix.

When apparatus "Basket" is used, place either one tablet or one capsule of the preparation to be tested in a dry basket at the beginning of each test. Lower the basket into position before rotation.

Immediately start rotation of the blade or basket at the rate specified in the individual monograph.

Withdraw a sample from a zone midway between the surface of the dissolution medium and the top of the rotating blade or basket, not less than 10 mm below the surface[1] and at least 10 mm from the vessel wall, at the time or time intervals specified (see under *Details of the procedure,* below).

Either replace the volume of dissolution medium with a volume equal to that of the liquid removed, or compensate for the loss of liquid by calculation, except where continuous measurement is used.

For filtration of the removed liquid, use an inert filter with a suitable pore size. Use a filter that does not cause significant adsorption of the active ingredient from the solution, and does not contain substances extractable by the dissolution medium that would interfere with the specified method of analysis. Use centrifugation as an alternative with conditions depending on the sample being tested.

Unless otherwise indicated, proceed in parallel with five additional tablets or capsules.

Determine the quantity of active ingredient dissolved in the specified time limit indicated in the individual monograph. The result should be expressed as a percentage of the content stated on the label.

Details of the procedure are given below for those not familiar with the method.

Details of the procedure

The following text is provided to give information and guidance, and is not regarded as an analytical requirement.

1. Verification and validation of equipment (six sets)

- Check the straightness of the shaft visually and with a ruler.
- Examine the paddle or basket for cracks in coating, if applicable.
- Check the paddle or basket for specified dimensions, particularly for any deviation in the evenness of the blade and its distance from the axis.
- Mount the paddle or basket and check its central position.
- Check the apparatus to ensure the desired rotation speed can be maintained within the limits ±4% throughout the test.
- Check the level of vibration of the whole apparatus, preferably measured with a vibration meter, and eliminate any sources of vibration to keep the readings of displacement below 0.3 mm.

[1] Recommended, 4.5 cm.

— Inspect the paddle or basket, and any portion of the apparatus which will be in contact with the test solutions, for cleanliness; in particular check the paddle/shaft joint for crevices or streaks.
— Inspect the vessels for cleanliness and for any abnormalities in dimension or shape, especially of the hemispherical bottom and internal radius. Place in the dissolution test bath.
— Insert the paddle or basket into the unit and adjust it to the specified distance from the bottom of the vessel (25 ± 2 mm).
— Adjust each vessel with a centring gauge. Mark the vessels to permit their easy replacement without losing the correct centred position.
— Calibrate the system with suitable calibrators,[1] according to a scheduled periodic system.

2. *Preparation of the dissolution medium*

— Select the dissolution medium (see section 6).
— Check the pH of the dissolution medium to two decimal places, using a suitably calibrated pH-meter.
— Dissolved gases can cause bubbles to form which may change the results of the test. In such cases, dissolved gases should be removed prior to testing using a suitable method (e.g. filtration under vacuum or in an ultrasonic water-bath).
— Preheat the medium to 37 °C or slightly above, measuring the temperature with precalibrated precision thermometers.

3. *Getting ready for the test*

3.1 Apparatus

— Adjust the speed to that prescribed in the monograph and ensure that it can be maintained within the limits ±4%.
— Ensure there is no significant wobble on any rotating shaft.
— Recheck the centring of the vessels with a gauge and correct if the tilt adjustments were altered.
— Set vertical frame limits, using markers or collars, in order to insert the sample to be tested without causing changes between each test.
— Prepare the test samples ready for dropping into the vessels.

3.2 Water-bath or other suitable heating system

— Transfer the dissolution medium, accurately measured to ±2%, to the vessel, check the temperature, and check the volumetric procedure used for subsequent determination as well as any weighing equipment employed.

[1] For example, calibrator tablets such as those available from United States Pharmacopeial Convention Inc.

— Use a transparent water-bath that allows for visual monitoring of the process of disintegration/desegregation, and the possible presence of air.
— Adjust the bath temperature to maintain a temperature of 37 ± 0.5 °C in the vessels.
— Ensure that the fluid in the bath is above the top level of the medium in the vessels.
— Ensure that the position of the water-bath is horizontal.

3.3 Sampling procedures

• Documentation

— Determine the intervals of sampling as given in the sampling procedure, and decide if a staggered or a simultaneous start for the six tests is convenient.
— Determine whether the time interval allowed between sampling is sufficient (especially for manual sampling).
— Record all information concerning:
  (a) the reference material used and how it is prepared;
  (b) the use of preevaluated correcting factors in the calculations for any interference from excipients, if known;
  (c) the preparation of samples for analysis: this must be checked to ensure that it does not interfere with the analysis, e.g. solvent/pH effects;
  (d) the pH of the solution to be tested and whether it is the same as that of the test medium used;
  (e) the diluent for the assay if it is not the same as the dissolution medium; and
  (f) the possible effects, if known, from other solvents.

• Inspection

— In manual sampling, check that the syringe or other sampling devices are clean.
— Observe that no interference occurs with the sampling probes.
— Ensure that the sample is withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating blade or basket, not less than 10 mm from the vessel wall, and that each subsequent sample is taken in the same way.

3.4 Selecting and checking analytical procedures

— Use the method specified in the corresponding monograph.
— The correct filters should be used to avoid adsorption or interference, unless separation is performed by centrifugation.
— Suitable tubing should be used in automated equipment to avoid adsorption.
— If a spectrophotometric method is used, it is preferable to operate in the linear range of absorption (extinction) values at the specified wavelength.

4. Carrying out the test

— Examine the system to ensure that no air bubbles are present.
— For simultaneous sampling, add the test sample to each of the vessels: immediately start the paddle/basket and stopwatch.
— For staggered sampling (paddle method), it may be necessary to add the test sample when the paddles are rotating. Drop the test sample as close as possible to the centre of the vessel and start the stopwatch immediately. In order to stop the test sample from floating, anchor it to the bottom of the vessel using a suitable device such as a wire or glass helix.
— Check the temperature of the water-bath and that of the dissolution medium at the beginning and at the end of the test and note on the check list (see also section 5).
— Proceed with the sampling.

5. At the end of the test

— Check the temperature of the medium in the vessels and record manually or automatically any deviations from the tolerance of ±0.5 °C.
— Record the speed of rotation.
— Ensure that all data are recorded or printed before discarding the samples (possible problems may only be solved by duplication).
— Note any unusual appearance, such as a silvery hue which indicates the release of dissolved gas.
— Note the condition of any undissolved part of the test sample, such as its position, form, etc. (This is valuable information for problem solving.)
— Check the volume of the contents of one or two vessels to ascertain if evaporation took place. This could affect the analytical results.

6. Suitable dissolution media

The preparation of suitable dissolution media is described above.

7. Checklist

The following checklist is offered as an example for the purpose of monitoring the performance of the system. It does not constitute a part of the requirements.

Proposed checklist for performance of the dissolution test
(one sheet per test)

Name of product
International Nonproprietary Name (INN) .....
Proprietary Name .....

Date .....

Spectrophotometer	**Verified**	**Further details (values and comments)**
Cleanliness of cells	.....	.....
Description		
Apparatus	.....	.....
Device(s)	.....	.....
– single/multi-spindle (3/6 vessels)	.....	.....
Cleanliness of paddle or basket	.....	.....
Cleanliness of vessel(s)	.....	.....
Dissolution medium (name and composition)	.....	
.....		
– pH of buffer	.....	.....
– de-aerated water	.....	.....
Filling of vessel and its position	.....	.....
Level of the dissolution medium	.....	
Position of paddle or basket (2.5 cm from the bottom of the vessel)	.....	
Insertion of tablets		
– time interval	.....	.....
– position	.....	.....
Initial speed	.....	
– after 15 minutes	.....	
– after 30 minutes	.....	
Initial temperature of dissolution medium	.....	
– after 15 minutes	.....	
– after 30 minutes	.....	
Sampling		
Cleanliness of withdrawal device (e.g. needle)	.....	
Initial position of device (e.g. 4.5 cm from the surface of the dissolution medium)	.....	.....
Position of device throughout the test	.....	.....

Results

Name of product
Date

Test sample	Percentage of active ingredient dissolved/time					
	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
1						
2						
3						
4						
5						
6						
Mean value						
Standard deviation						
Relative standard deviation (%)						

5.6 Test for extractable volume for parenteral preparations

The determination of extractable volume applies to the following preparations for parenteral use: injections and parenteral infusions.

Recommended procedures

Injections

The volume of the injection in a single-dose container is usually sufficient to permit withdrawal of the nominal dose. The single-dose container does not hold a quantity relative to the declared volume that would present a risk should the whole contents be administered.

Compliance with the requirement for extractable volume is assured by making the filling volume greater than the nominal volume to a degree determined by the characteristics of the product.

Suspensions and emulsions must be shaken prior to withdrawal of the contents and before proceeding with the determination of the density.

Oily or viscous preparations may need to be heated and thoroughly shaken immediately before withdrawal of the contents.

Test for containers with a nominal volume of less than 5 ml

Take five containers. Choose a syringe with a capacity not greater than twice the volume to be measured, fitted with a suitable needle.

Withdraw as much of the contents as possible from one of the five containers to be used in the test, expel any bubbles and transfer this quantity, without emptying the needle, into a dry tared container. Weigh the whole and determine the mass of the contents. Repeat the procedure with the four remaining containers.

Determine the density of the preparation at the temperature at which the test is carried out. From the mass of the contents of each container, calculate the corresponding volume by dividing by the density.

The preparation complies with the test for extractable volume if the volume measured for each of the five containers is not less than its nominal value.

Test for containers with a nominal volume of 5 ml or more

Take five containers. Choose a syringe with a capacity not greater than twice the volume to be measured, fitted with a suitable needle.

Withdraw as much of the contents as possible from one of the five containers to be used in the test, expel any bubbles and transfer this quantity, without emptying the needle, into a dry measuring cylinder with a capacity such that the volume to be measured is not less than 40% of the nominal volume of the cylinder. Measure the volume transferred. Repeat the procedure with the four remaining containers.

The preparation complies with the test for extractable volume if the volume measured for each of the five containers is not less than its nominal value.

Parenteral infusions

Take one container. Transfer its contents into a dry measuring cylinder with a capacity such that the volume to be measured is not less than 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume measured is not less than the nominal value stated on the container.

5.7 Visual inspection of particulate matter in injectable preparations

Particulate contamination of injections and parenteral infusions consists of extraneous, mobile, undissolved particles unintentionally present in the solutions. Disregard any gas bubbles.

The types of preparation for which compliance with this test is required are stated in the individual monograph.

This test provides a simple method for the detection of visible particles. It is performed in accordance with the provisions of Good Manufacturing Practices. The test is not intended for use by a manufacturer for batch release purposes. To ensure that a product will meet pharmacopoeial specifications with respect to visible particulate matter, if and when tested, manufacturers should

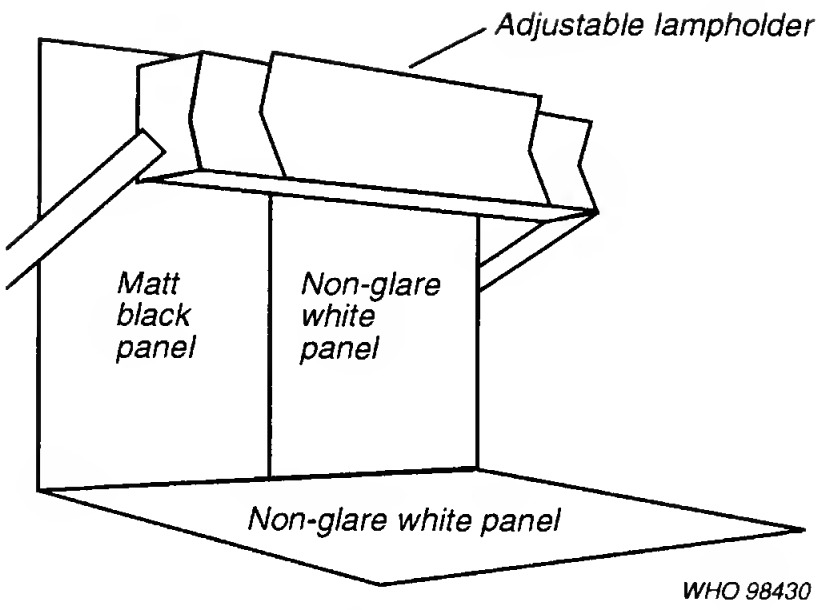

Figure 5. Apparatus for visible particles
Reproduced with the permission of the European Pharmacopoeia Commission, European Directorate for the Quality of Medicines, Council of Europe.

carry out a 100% inspection and rejection of unsatisfactory items prior to release or use other appropriate means.

Subvisible particles and the nature of the particles are not identified by this method.

Apparatus[1]

The apparatus (Fig. 5) consists of a viewing station comprising:

- — a matt black panel of appropriate size held in a vertical position;
- — a non-glare white panel of appropriate size held in a vertical position next to the black panel;
- — an adjustable lampholder fitted with a shaded, white-light source and with a light diffuser (a viewing illuminator containing two 13-W fluorescent tubes, each 525 mm in length is suitable). The intensity of illumination at the viewing point is maintained between 2000 lux and 3750 lux for clear glass ampoules. Higher values are preferable for coloured glass and plastic containers.

[1] This method was developed by WHO in collaboration with Group 12 of the European Pharmacopoeia Commission.

Recommended procedure

Gently swirl or invert each individual container, making sure that no air bubbles are introduced, and observe for about 5 seconds in front of the white panel. Repeat the procedure in front of the black panel.

Record the presence of any particles. Repeat the procedure for a further 19 containers.

The preparation fails the test if one or more particles are found in more than one container.

When the test is applied to reconstituted solutions from powder for injections, the test fails if particles are found in more than two containers.

5.8 Methods of sterilization

Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoa) that could contaminate pharmaceuticals or other materials and thereby constitute a health hazard. Since the achievement of the absolute state of sterility cannot be demonstrated, the sterility of a pharmaceutical preparation can be defined only in terms of probability. The efficacy of any sterilization process will depend on the nature of the product, the extent and type of any contamination, and the conditions under which the final product has been prepared. The requirements for Good Manufacturing Practice should be observed throughout all stages of manufacture and sterilization.

Classical sterilization techniques using saturated steam under pressure or hot air are the most reliable and should be used whenever possible. Other sterilization methods include filtration, ionizing radiation (gamma and electron-beam radiation), and gas (ethylene oxide, formaldehyde).

For products that cannot be sterilized in the final containers, aseptic processing is necessary. Materials and products that have been sterilized by one of the above processes are transferred to presterilized containers and sealed, both operations being carried out under controlled aseptic conditions.

Whatever method of sterilization is chosen, the procedure must be validated for each type of product or material, both with respect to the assurance of sterility and to ensure that no adverse change has taken place within the product. Failure to follow precisely a defined, validated process could result in a non-sterile or deteriorated product. A typical validation programme for steam or dry-heat sterilization requires the correlation of temperature measurements, made with sensory devices to demonstrate heat penetration and heat distribution, with the destruction of biological indicators, i.e. preparations of specific microorganisms known to have high resistance to the particular sterilization process. Biological indicators are also used to validate other sterilization methods (see specific methods), and sometimes for routine control of individual cycles. Periodic revalidation is recommended.

Heating in an autoclave (steam sterilization)

Exposure of microorganisms to saturated steam under pressure in an autoclave achieves their destruction by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. As displacement of the air by steam is unlikely to be readily achieved, the air should be evacuated from the autoclave before admission of steam. This method should be used whenever possible for aqueous preparations and for surgical dressings and medical devices.

The recommendations for sterilization in an autoclave are 15 minutes at 121–124 °C (200 kPa).[1] The temperature should be used to control and monitor the process; the pressure is mainly used to obtain the required steam temperature. Alternative conditions, with different combinations of time and temperature, are given below.

Temperature (°C)	Approximate corresponding pressure (kPa)	Minimum sterilization time (min)
126–129	250 (~2.5 atm)	10
134–138	300 (~3.0 atm)	5

Minimum sterilization time should be measured from the moment when all the materials to be sterilized have reached the required temperature throughout. Monitoring the physical conditions within the autoclave during sterilization is essential. To provide the required information, temperature-monitoring probes should be inserted into representative containers, with additional probes placed in the load at the potentially coolest parts of the loaded chamber (as established in the course of the validation programme). The conditions should be within ±2 °C and ±10 kPa (±0.1 atm) of the required values. Each cycle should be recorded on a time-temperature chart or by other suitable means.

Aqueous solutions in glass containers usually reach thermal equilibrium within 10 minutes for volumes up to 100 ml and 20 minutes for volumes up to 1000 ml.

Porous loads, such as surgical dressings and related products, should be processed in an apparatus that ensures steam penetration. Most dressings are adequately sterilized by maintaining them at a temperature of 134–138 °C for 5 minutes.

In certain cases, glass, porcelain, or metal articles are sterilized at 121–124 °C for 20 minutes.

Fats and oils may be sterilized at 121 °C for 2 hours but, whenever possible, should be sterilized by dry heat.

[1] 1 atm = 101 325 Pa

In certain cases (e.g. thermolabile substances), sterilization may be carried out at temperatures below 121 °C, provided that the chosen combination of time and temperature has been validated. Lower temperatures offer a different level of sterilization; if this is evaluated in combination with the known microbial burden of the material before sterilization, the lower temperatures may be satisfactory. Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The bioindicator strain proposed for validation of this sterilization process is: spores of *Bacillus stearothermophilus* (e.g. ATCC 7953 or CIP 52.81) for which the D-value (i.e. 90% reduction of the microbial population) is 1.5–2 minutes at 121 °C, using about 10^6 spores per indicator.

Dry-heat sterilization

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. Dry-heat sterilization requires a higher temperature than moist heat and a longer exposure time. The method is, therefore, more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate. Such materials include glassware, powders, oils, and some oil-based injectables.

Preparations to be sterilized by dry heat are filled in units that are either sealed or temporarily closed for sterilization. The entire content of each container is maintained in the oven for the time and at the temperature given in the table below. Other conditions may be necessary for different preparations to ensure the effective elimination of all undesirable microorganisms.

Temperature (°C)	Minimum sterilization time (min)
160	180
170	60
180	30

Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The oven should normally be equipped with a forced air system to ensure even distribution of heat throughout all the materials processed. This should be controlled by monitoring the temperature. Containers that have been temporarily closed during the sterilization procedure are sealed after sterilization using aseptic techniques to prevent microbial recontamination.

The bioindicator strain proposed for validation of the sterilization process is: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) for which the D-value is 5–10 minutes at 160 °C using about 10^6 spores per indicator.

Filtration

Sterilization by filtration is employed mainly for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Asbestos-containing filters should not be used.

Appropriate measures should be taken to avoid loss of solute by adsorption onto the filter and to prevent the release of contaminants from the filter. Suitable filters will prevent the passage of microorganisms, but the filtration must be followed by an aseptic transfer of the sterilized solution to the final containers which are then immediately sealed with great care to exclude any recontamination.

Usually, membranes of not greater than 0.22 µm nominal pore size should be used. The effectiveness of the filtration method must be validated if larger pore sizes are employed.

To confirm the integrity of filters, both before and after filtration, a bubble point or similar test should be used, in accordance with the filter manufacturer's instructions. This test employs a prescribed pressure to force air bubbles through the intact membrane previously wetted with the product, with water, or with a hydrocarbon liquid.

All filters, tubes, and equipment used "downstream" must be sterile. Filters capable of withstanding heat may be sterilized in the assembly before use by autoclaving at 121 °C for 15–45 minutes depending on the size of the filter assembly. The effectiveness of this sterilization should be validated. For filtration of a liquid in which microbial growth is possible, the same filter should not be used for procedures lasting longer than one working day.

Exposure to ionizing radiation

Sterilization of certain active ingredients, drug products, and medical devices in their final container or package may be achieved by exposure to ionizing radiation in the form of gamma radiation from a suitable radioisotopic source such as ^{60}Co (cobalt 60) or of electrons energized by a suitable electron accelerator. Laws and regulations for protection against radiation must be respected.

Gamma radiation and electron beams are used to effect ionization of the molecules in organisms. Mutations are thus formed in the DNA and these reactions alter replication. These processes are very dangerous and only well-trained and experienced staff should decide upon the desirability of their use and should ensure monitoring of the processes. Specially designed and purpose-built installations and equipment must be used.

It is usual to select an absorbed radiation level of 25 kGy[1] (2.5 Mrad)[2], although other levels may be employed provided that they have been validated.

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[1] kilogray
[2] megarad

Radiation doses should be monitored with specific dosimeters during the entire process. Dosimeters should be calibrated against a standard source on receipt from the supplier and at appropriate intervals thereafter. The radiation system should be reviewed and validated whenever the source material is changed and, in any case, at least once a year.

The bioindicator strains proposed for validation of this sterilization process are: spores of *Bacillus pumilus* (e.g. ATCC 27142 or CIP 77.25) with 25 kGy (2.5 Mrad) for which the D-value is about 3 kGy (0.3 Mrad) using 10^7–10^8 spores per indicator; for higher doses, spores of *Bacillus cereus* (e.g. SSI C 1/1) or *Bacillus sphaericus* (e.g. SSI C_1A) are used.

Gas sterilization

The active agent of the gas sterilization process can be ethylene oxide or another highly volatile substance. The highly flammable and potentially explosive nature of such agents is a disadvantage unless they are mixed with suitable inert gases to reduce their highly toxic properties and the possibility of toxic residues remaining in treated materials. The whole process is difficult to control and should only be considered if no other sterilization procedure can be used. It must only be carried out under the supervision of highly skilled staff.

The sterilizing efficiency of ethylene oxide depends on the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. In particular, it is necessary to ensure that the nature of the packaging is such that the gas exchange can take place. It is also important to maintain sufficient humidity during sterilization. Records of gas concentration and of temperature and humidity should be made for each cycle. Appropriate sterilization conditions must be determined experimentally for each type of load.

After sterilization, time should be allowed for the elimination of residual sterilizing agents and other volatile residues, which should be confirmed by specific tests.

Because of the difficulty of controlling the process, efficiency must be monitored each time using the proposed bioindicator strains: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372 or CIP 77.18) or of *Bacillus stearothermophilus*, (e.g. ATCC 7953 or CIP 52.81). The same quantity of spores should be used as for "Heating in an autoclave" and "Dry-heat sterilization".

Reagents, test solutions and volumetric solutions

Reagents, test solutions and volumetric solutions

The reagents, test solutions and volumetric solutions mentioned in the *International Pharmacopoeia,* 4th edition, are described below. The reagents are denoted by the abbreviation R, the test solutions by the abbreviation TS, and the volumetric solutions, or solutions that are similarly standardized, by the abbreviation VS. Similarly named reagents differing in composition, purity, etc., are distinguished by placing a numeral after the appropriate abbreviation. The designations AsR, AsTS, ClTS, FeTS and PbTS refer to reagents of suitable purity for use in the limit tests for arsenic, chlorides, iron, and heavy metals, respectively. The designation IR refers to reagents of suitable purity for use in spectrophotometry in the infrared region. The designation Cm denotes culture media for microbiological tests. The designation RS denotes International Chemical Reference Substances.[1]

The concentrations are expressed in conformity with the *Système international d'Unités* (SI) and they refer to the anhydrous substance. The reference to SRIP indicates *Specifications for reagents mentioned in the International Pharmacopoeia* (World Health Organization, Geneva, 1963). Designations used in SRIP but now discontinued are given in square brackets. The designation *d* denotes the relative density d_{20}^{20}, i.e., measured in air at 20 °C in relation to water at 20 °C.

Unless otherwise specified, all solutions indicated in the tests and assays of *The International Pharmacopoeia* are prepared with water R.

Acacia (5 g/l) TS. A solution in water containing about 5 g of acacia R per litre.

Acacia R. The dried gummy exudate from the stems and branches of *Acacia senegal* (L.) Willd. and of other species of *Acacia* of African origin.

Description. Rounded or ovoid tears of varying diameters from about 1 cm to 3 cm; yellowish white or pale amber; odourless.

Solubility. Very slowly soluble in twice its weight of water, leaving only a very small residue of vegetable particles; practically insoluble in ethanol (~750 g/l) TS and ether R.

Ash. Not more than 50 mg/g.

Acid-insoluble ash. Not more than 5.0 mg/g.

Insoluble matter. Mix 5 g of powdered or finely ground material with 100 ml of water and 10 ml of hydrochloric acid (~70 g/l) TS and boil gently for 15 minutes, stirring frequently. Filter while hot through a sintered glass cru-

[1] *International Chemical Reference Substances are available from the WHO Collaborating Centre for Chemical Reference Substances, Apoteket AB, Produktion & Laboratorier, Centrallaboratoriet, ACL, Prismavägen 2, S-141 75 Kungens Kurva, Sweden. (Fax: +46 8 740 60 40; email:who.apl@apoteket.se).*

cible, wash the residue with hot water, and dry to constant weight at 105 °C; not more than 5 mg/g.
Tannin. Dissolve 1 g in 10 ml of water and add 0.1 ml of ferric chloride (25 g/l) TS; no bluish black colour or blackish precipitate is produced.

Acetaldehyde R. Ethanal; C_2H_4O.
Description. A clear, colourless, flammable liquid.
Miscibility. Miscible with water and ethanol (~750 g/l) TS.
Refractive index. $n_D^{20} = 1.332$
Relative density. $d_{20}^{20} = 0.788$
Boiling point. About 21 °C.

p-Acetamidobenzalazine RS. International Chemical Reference Substance.

Acetate buffer, pH 3.0, TS. A buffer mixture of pH 3.0.
Procedure. Dissolve 12 g of sodium acetate R in water, add 6 ml of glacial acetic acid R and dilute with sufficient water to produce 100 ml.

Acetate buffer, pH 4.5, TS
Procedure. Dissolve 10.9 g of sodium acetate R in 100 ml of water, add 8 ml of glacial acetic acid R, mix, and dilute to 1000 ml with water.

Acetate buffer, pH 4.6, TS
Procedure. Dissolve 5.4 g of sodium acetate R in 50 ml of water, adjust to pH 4.6 with glacial acetic acid R, and dilute to 100 ml with water.

Acetate buffer, pH 4.7, TS
Procedure. Dissolve 8.4 g of sodium acetate R in 100 ml of water, add 3.35 ml of glacial acetic acid R, mix, and dilute to 1000 ml with water.

Acetate buffer, pH 5.0, TS
Procedure. Dissolve 13.6 g of sodium acetate R in 100 ml of water, add 6 ml of glacial acetic acid R, mix, and dilute to 1000 ml with water.

Acetate buffer, pH 5.5, TS
Procedure. Dissolve 54.4 g of sodium acetate R in 50 ml of water, heating to 35 °C, if necessary. After cooling, slowly add 10 ml of glacial acetic acid R. Shake and dilute to 1000 ml with water.

Acetate buffer, pH 6.0, TS
Procedure. Dissolve 100 g of ammonium acetate R in 300 ml of water. Add 4.1 ml of glacial acetic acid R, adjust the pH to 6.0 using either ammonia (~100 g/l) TS or acetic acid (~300 g/l) TS, and dilute to 500 ml with water.

Acetate standard buffer TS

Procedure. To 10 ml of acetic acid (~60 g/l) TS add 10 ml of sodium hydroxide (1 mol/l) VS and dilute with sufficient carbon-dioxide-free water R to produce 1000 ml.

Acetazolamide RS. International Chemical Reference Substance.

Acetic acid (~90 g/l) TS. Acetic acid (~300 g/l) TS, diluted with water to contain about 90 g of $C_2H_4O_2$ per litre (approximately 1.5 mol/l).

Acetic acid (~120 g/l) TS. Acetic acid (~300 g/l) TS, diluted with water to contain 120 g of $C_2H_4O_2$ per litre (approximately 2 mol/l); d ~ 1.016.

Acetic acid (~300 g/l) TS. A solution of glacial acetic acid R containing about 300 g/l of $C_2H_4O_2$ (approximately 5 mol/l); d ~ 1.037.

Acetic acid (~60 g/l) PbTS. Acetic acid (~60 g/l) TS that complies with the following test: Evaporate 20 ml of acetic acid (~60 g/l) TS almost to dryness on a water-bath, add 25 ml of water and carry out the test for heavy metals. The heavy metals limit is 3 µg/ml.

Acetic acid (~60 g/l) TS. Acetic acid (~300 g/l) TS, diluted to contain about 60 g/l of $C_2H_4O_2$ (approximately 1 mol/l); d ~ 1.008.

Acetic acid (0.07 mol/l) VS. A solution prepared by diluting 4.2 ml of glacial acetic acid R to 1000 ml with water.

Acetic acid (5.0 g/l) TS. Acetic acid (~300 g/l) TS, diluted with water to contain about 5.0 g of $C_2H_4O_2$ per litre; d ~ 1.0007.

Acetic acid, glacial, R. $C_2H_4O_2$ (SRIP, 1963, p. 25); d ~ 1.048.

Acetic acid, glacial, R1. Glacial acetic acid R, that complies with the following tests:

Substances reducing dichromate. To 10 ml add 1.0 ml of potassium dichromate (0.0167 mol/l) VS and cautiously add 10 ml of sulfuric acid (~1760 g/l) TS. Cool the solution to room temperature and allow to stand for 30 minutes. While swirling the solution, dilute slowly and cautiously with 50 ml of water, cool, and add 1.5 ml of freshly prepared potassium iodide (80 g/l) TS. Titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, adding 3 ml of starch TS as the endpoint is approached. Perform a blank titration and make any necessary corrections. Not less than 0.60 ml of sodium thiosulfate (0.1 mol/l) VS is consumed.

Substances reducing permanganate. Add 40 ml to 10 ml of water. Cool to 15 °C, add 0.30 ml of potassium permanganate (0.02 mol/l) VS, and allow to stand at 15 °C for 10 minutes; the pink colour is not entirely discharged.

Acetic anhydride R. $C_4H_6O_3$ (SRIP, 1963, p. 26).

Acetic anhydride/dioxan TS
Procedure. To 50 ml of dioxan R add 1 ml of acetic anhydride R (approximately 0.2 mol/l).

Acetone R. C_3H_6O (SRIP, 1963, p. 27).

Acetonitrile (400 g/l) TS
Procedure. Mix 1 volume of acetonitrile R with 1 volume of water. The resulting solution contains about 400 g/l of C_2H_3N.

Acetonitrile R. Methyl cyanide, C_2H_3N.
Description. A clear, colourless liquid.
Miscibility. Freely miscible with water.

Acetyl chloride R. C_2H_3ClO. Contains not less than 98.0% of C_2H_3ClO in both assay A and assay B (see below).
Description. A clear, colourless or very slightly yellow liquid.
Phosphorus compounds. Carefully treat 1 ml with 1 ml of water, add 1 ml of nitric acid (~1000 g/l) TS, boil, cool, dilute with 20 ml of water, add 10 ml of ammonium molybdate/nitric acid TS and allow to stand at about 40 °C for 2 hours; no yellow precipitate is produced.
Assay. (A) Dissolve about 1 g, accurately weighed, in 50 ml of carbonate-free sodium hydroxide (1 mol/l) VS, and titrate with sulfuric acid (0.5 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 7.850 mg of C_2H_3ClO. (B) Dilute the neutralized liquid from A to 250 ml with water, mix, and titrate 50 ml with silver nitrate (0.1 mol/l) VS, using potassium chromate (100 g/l) TS as indicator. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 7.850 g of C_2H_3ClO.

Agar R. (SRIP, 1963, p. 27).

Albendazole RS. International Chemical Reference Substance.

Alcuronium chloride RS. International Chemical Reference Substance.

Allopurinol RS. International Chemical Reference Substance.

Alum R. Aluminium potassium sulfate dodecahydrate; $KAl(SO_4)_2$, $12H_2O$ (SRIP, 1963, p. 29).

Aluminium chloride R. $AlCl_3,6H_2O$ (SRIP, 1963, p. 30).

Aluminium chloride TS

Procedure. Dissolve 65.0 g of aluminium chloride R in sufficient water to produce 100 ml, add 0.5 g of charcoal R, stir for 10 minutes and filter. While stirring, add to the filtrate sufficient sodium hydroxide (0.5 mol/l) VS to adjust the pH to 1.5.

Aluminium hydroxide R. Hydrated $Al(OH)_3$.

Description. A white, odourless powder.

Solubility. Practically insoluble in water and in ethanol (~750 g/l) TS.

Aluminium oxide R. Al_2O_3.

A suitable grade for use in thin-layer chromatography.

Aluminium R. Al (SRIP, 1963, p. 29); wire, granules, or sheets.

Aluminium standard (10 µg Al/ml) TS

Procedure. Dissolve 17.6 mg of alum R in 5 ml of sulfuric acid (0.05 mol/l) VS and dilute to 100 ml with water.

Note. For the preparation of this test solution commercially available aluminium nitrate standard solution 1000 µg Al^{3+}/ml or aluminium nitrate nonahydrate can also be used.

Amidotrizoic acid RS. International Chemical Reference Substance.

Amiloride hydrochloride RS. International Chemical Reference Substance.

3-Amino-2,4,6-triiodobenzoic acid RS. International Chemical Reference Substance.

2-Amino-5-nitrothiazole R. $C_3H_3N_3O_2S$.

Description. Greenish yellow to orange-yellow, fluffy powder.

Solubility. Very slightly soluble in water; soluble in dilute mineral acids; slightly soluble in ethanol (~750 g/l) TS and ether R.

Melting temperature. About 198 °C with decomposition.

4-Amino-6-chloro-1,3-benzenedisulfonamide R. $C_6H_8ClN_3O_4S_2$.

Description. A white, odourless powder.

Solubility. Soluble in ammonia (~100 g/l) TS; practically insoluble in water.

Identification. The absorption spectrum of a 5 µg/ml solution in methanol R exhibits maxima at about 223 nm, 265 nm, and 312 nm. The absorptivity at 265 nm is about 64.0 ($E^{1\%}_{1cm}$ = 640).

Sulfated ash. Ignite 2 g; not more than 1.0 mg/g.

4-Aminoantipyrine R. $C_{11}H_{13}N_3O$. 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one; ampyrone; aminopyrazolone.

Description. Pale yellow crystals or powder.

Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.
Melting temperature. About 108 °C.

4-Aminoantipyrine TS1
Procedure. Dissolve 0.125 g of 4-aminoantipyrine R in 25 ml of methanol R containing 0.25 ml of hydrochloric acid (~420 g/l) TS.

4-Aminoantipyrine TS2
Procedure. Dissolve about 0.1 g of 4-aminoantipyrine R in 30 ml of water and add a mixture of 10 ml of sodium carbonate (200 g/l) TS and 2 ml of sodium hydroxide (1 mol/l) VS; dilute with sufficient water to produce 100 ml.
Note: 4-Aminoantipyrine TS2 must be freshly prepared.

4-Aminobenzoic acid R. $C_7H_7NO_2$. Contains not less than 98.5% of $C_7H_7NO_2$.
Description. White or slightly yellow crystals or a crystalline powder; odourless.
Solubility. Soluble in 170 parts of water, in 9 parts of boiling water, in 8 parts of ethanol (~750 g/l) TS, and in 50 parts of ether R.
Melting range. 186–189 °C.
Sulfated ash. Not more than 1.0 mg/g.
Loss on drying. Dry at 105 °C for 2 hours; it loses not more than 2.0 mg/g.
Assay. Transfer to a beaker about 0.3 g, accurately weighed and previously dried at 105 °C for 2 hours, add 5 ml of hydrochloric acid (~420 g/l) TS, 50 ml of water and stir until dissolved. Cool to about 15 °C, add about 25 g of crushed ice and slowly titrate with sodium nitrite (0.1 mol/l) VS until a glass rod dipped into the titrated solution produces an immediate blue ring when touched to starch/iodide paper R. When the titration is complete, the end-point is reproducible after the mixture has been allowed to stand for 1 minute. Each ml of sodium nitrite (0.1 mol/l) VS is equivalent to 13.71 mg of $C_7H_7NO_2$.
Storage. Store in a tightly closed container, protected from light.

2-Aminobutanol R. $C_4H_{11}NO$.
Description. A colourless or light yellow, clear liquid.
Miscibility. Miscible with water and methanol R.
Mass density. $\rho_{20} = 0.944 - 0.950$ kg/l.
Refractive index. $n_D^{20} = 1.450 - 1.455$.
Identification. Dissolve 0.05 g in 4 ml of ethanol (~750 g/l) TS, add 0.5 ml of a 2.5 mg/ml solution of triketohydrindene hydrate R and warm on a water-bath; a violet colour is produced.

7-[(2-Aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid RS (ciprofloxacin ethylenediamine compound). International Chemical Reference Substance.

4-Aminophenol R. C_6H_7NO.
Description. A white or almost white, crystalline powder.
Melting temperature. About 184 °C with decomposition.

3-Aminopyrazole-4-carboxamide hemisulfate RS. International Chemical Reference Substance.

Amitriptyline hydrochloride RS. International Chemical Reference Substance.

Ammonia (~100 g/l) FeTS. Ammonia (~100 g/l) TS that complies with the following test: Evaporate 5 ml of ammonia (~100 g/l) TS nearly to dryness on a water-bath, add 40 ml of water, 2 ml of citric acid (180 g/l) FeTS, and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/l) FeTS, and dilute to 50 ml with water; no pink colour is produced.

Ammonia (~100 g/l) PbTS. Ammonia (~100 g/l) TS that complies with the following test: Evaporate 5 ml of ammonia (~100 g/l) TS to dryness on a water-bath, add to the residue 1 ml of hydrochloric acid (~70 g/l) TS, and evaporate to dryness. Dissolve the residue in 2 ml of acetic acid (~60 g/l) PbTS, dilute with water to 25 ml and carry out the test for heavy metals. Prepare the blank in a similar way. The heavy metals limit is 2 µg/ml.

Ammonia (~100 g/l) TS. Ammonia (~260 g/l) TS, diluted to contain about 100 g/l of NH_3 (approximately 6 mol/l); d ~ 0.956.

Ammonia (~17 g/l) TS. Ammonia (~100 g/l) TS, diluted to contain about 17 g of NH_3 per litre (approximately 1 mol/l); d ~ 0.992.

Ammonia (~260 g/l) TS. [ammonia, strong, R]. (SRIP, 1963, p. 31); d ~ 0.894.

Ammonia (~35 g/l) TS. Ammonia (~100 g/l) TS, diluted to contain about 35 g of NH_3 per litre (approximately 2 mol/l); d ~ 0.985.

Ammonia (~50 g/l) TS. Ammonia (~260 g/l) TS, diluted with water to contain about 50 g of NH_3 per litre (approximately 3 mol/l); d ~ 0.977.

Ammonia buffer TS
Procedure. Dissolve 67.5 g of ammonium chloride R in 570 ml of ammonia (~260 g/l) TS and dilute with water to 1000 ml.

Ammonia buffer TS2
Procedure. Dissolve 67.5 g of ammonium chloride R in 650 ml of ammonia (~260 g/l) TS and dilute with water to produce 1000 ml.

Ammonium acetate (100 g/l) TS. A solution of ammonium acetate R containing 100 g of $C_2H_7NO_2$ per litre.

Ammonium acetate (2 g/l) TS. A solution of ammonium acetate R containing about 2 g of $C_2H_7NO_2$ per litre.
Note: Ammonium acetate (2 g/l) TS must be freshly prepared.

Ammonium acetate (40 g/l) TS. A solution of ammonium acetate R containing about 38.5 g of $C_2H_7NO_2$ per litre (approximately 0.5 mol/l).
Note: Ammonium acetate (40 g/l) TS must be freshly prepared.

Ammonium acetate (50 g/l) TS. A solution of ammonium acetate R containing about 50 g of $C_2H_7NO_2$ per litre.

Ammonium acetate (80 g/l) TS. A solution of ammonium acetate R containing about 77 g/l of $C_2H_7NO_2$ (approximately 1 mol/l).

Ammonium acetate TS
Procedure. Dissolve 150 g of ammonium acetate R in water, add 3 ml of glacial acetic acid R, and dilute with sufficient water to produce 1000 ml.
Note: Ammonium acetate TS must be used within 1 week of preparation.

Ammonium acetate buffer, pH 4.62, TS
Procedure. Adjust the pH of ammonium acetate (100 g/l) TS to 4.62 using acetic acid (~60 g/l) TS.

Ammonium acetate R. $C_2H_7NO_2$ (SRIP, 1963, p. 32).

Ammonium carbonate R. $(NH_4)_2CO_3$ (SRIP, 1963, p. 33).

Ammonium chloride (10 µg/ml NH_4) TS
Procedure. Dissolve 0.296 g, accurately weighed, of ammonium chloride R in sufficient water to produce 1000 ml. Dilute 10 ml of this solution to 100 ml.
Shelf-life. Use the solution within 2 weeks of its preparation.

Ammonium chloride (100 g/l) TS. A solution of ammonium chloride R containing about 100 g of NH_4Cl per litre.

Ammonium chloride (20 g/l) TS. A solution of ammonium chloride R containing about 20 g of NH_4Cl per litre.

Ammonium chloride buffer, pH 10.0, TS. A buffer mixture of pH 10.0.
Procedure. Dissolve 7.0 g of ammonium chloride R in 57 ml of ammonia (~260 g/l) TS and dilute with sufficient water to produce 100 ml.

Ammonium chloride buffer, pH 10.5, TS. A buffer mixture of pH 10.5.
Procedure. Dissolve 6.95 g of ammonium chloride R in 75 ml of ammonia (~260 g/l) TS and dilute to 100 ml with water.

Ammonium chloride R. NH_4Cl (SRIP, 1963, p. 33).

Ammonium chloride TS (Nessler's reagent)
Procedure. Dissolve 3.15 g of ammonium chloride R in a sufficient quantity of ammonia-free water R to produce 1000 ml.

Ammonium chloride, dilute, TS
Procedure. To 10 ml of ammonium chloride TS add a sufficient quantity of ammonia-free water R to produce 1000 ml.

Ammonium dihydrogen phosphate. $(NH_4)H_2PO_4$

Ammonium mercurithiocyanate TS
Procedure. Dissolve 30 g of ammonium thiocyanate R and 27 g of mercuric chloride R in sufficient water to produce 1000 ml.

Ammonium molybdate (45 g/l) TS. A solution of ammonium molybdate R containing about 47 g of $(NH_4)_6Mo_7O_{24}$ per litre.

Ammonium molybdate (95 g/l) TS. A solution of ammonium molybdate R containing about 95 g/l of $(NH_4)_6Mo_7O_{24}$.

Ammonium molybdate R. $(NH_4)_6Mo_7O_{24},4H_2O$ (SRIP, 1963, p. 34).

Ammonium molybdate/nitric acid TS
Procedure. Dissolve 50 g of ammonium sulfate R in 500 ml of nitric acid (~1000 g/l) TS using a 2000-ml conical flask or beaker. Dissolve separately in a beaker 150 g of ammonium molybdate R in 400 ml of boiling water. After cooling, pour this solution slowly, while stirring, into the acid solution and dilute with water to 1000 ml. Allow to stand for 2–3 days, and filter.
Storage. Store in well-closed, brown glass bottles, and keep in a cool place.

Ammonium molybdate/sulfuric acid TS
Procedure. Dissolve 0.5 g of ammonium molybdate R in sufficient sulfuric acid (~1760 g/l) TS to produce 10 ml.

Ammonium molybdate/vanadate TS
Procedure. Shake 4 g of finely powdered ammonium molybdate R and 0.1 g of finely powdered ammonium vanadate R with 70 ml of water. Add 20 ml of nitric acid (~1000 g/l) TS and dilute to 100 ml with water.

Ammonium nitrate (50 g/l) TS. A solution of ammonium nitrate R containing about 50 g of NH_4NO_3 per litre.

Ammonium nitrate R. NH_4NO_3 (SRIP, 1963, p. 35).

Ammonium nitrate TS

Procedure. Dissolve 1.6 g of ammonium nitrate R in 30 ml of water, add 3.0 ml of ammonia (~260 g/l) TS, and dilute with sufficient water to produce 100 ml.

Ammonium oxalate (25 g/l) TS. A solution of ammonium oxalate R containing about 27 g/l of $C_2H_8N_2O_4$.

Ammonium oxalate (50 g/l) TS. A solution of ammonium oxalate R containing about 50 g of $C_2H_8N_2O_4$ per litre.

Ammonium oxalate R. $C_2H_8N_2O_4,H_2O$ (SRIP, 1963, p. 36).

Ammonium persulfate R. $(NH_4)_2S_2O_8$ (SRIP, 1963, p. 37).

Ammonium persulfate/phosphate buffer TS

Procedure. Dissolve 10 g of ammonium persulfate R in sufficient phosphate buffer, pH 7.4, TS to produce 100 ml.

Ammonium pyrrolidinedithiocarbamate (10 g/l) TS

Procedure. Immediately before use, wash 10 g of ammonium pyrrolidinedithiocarbamate R three times, each with 25 ml of isobutyl methyl ketone R, filter, and dry the substance. Then dissolve 1.0 g in sufficient water to produce 100 ml.

Ammonium pyrrolidinedithiocarbamate R. Ammonium 1-pyrrolidinecarbodithioate; $C_5H_{12}N_2S_2$. Reagent grade quality.

Ammonium reineckate (10 g/l) TS. A solution of ammonium reineckate R containing about 10 g of $NH_4[Cr(NH_3)_2(SCN)_4]$ per litre.

Ammonium reineckate R. $NH_4[Cr(NH_3)_2(SCN)_4],H_2O$ (SRIP, 1963, p. 39).

Ammonium sulfamate (25 g/l) TS. A solution of ammonium sulfamate R containing about 25 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate (5 g/l) TS. A solution of ammonium sulfamate R containing about 5 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate (50 g/l) TS. A solution of ammonium sulfamate R containing 50 g of $NH_4OSO_2NH_2$ per litre.

Ammonium sulfamate R. $NH_4OSO_2NH_2$ (SRIP, 1963, p. 39).

Ammonium sulfate R. $(NH_4)_2SO_4$ (SRIP, 1963, p. 40).

Ammonium sulfide TS

Procedure. Prepare a saturated solution of hydrogen sulfide R in ammonia (~100 g/l) TS. To 25 ml of this solution add 50 ml of ammonia (~100 g/l) TS.

Ammonium thiocyanate (0.01 mol/l) VS. Ammonium thiocyanate R, dissolved in water to contain 0.7612 g of NH_4SCN in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under ammonium thiocyanate (0.1 mol/l) VS.

Ammonium thiocyanate (0.05 mol/l) VS. Ammonium thiocyanate R, dissolved in water to contain 3.806 g of NH_4SCN in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described for ammonium thiocyanate (0.1 mol/l) VS.

Ammonium thiocyanate (0.1 mol/l) VS. Ammonium thiocyanate R, dissolved in water to contain 7.612 g of NH_4SCN in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Place 30.0 ml of silver nitrate (0.1 mol/l) VS in a glass-stoppered flask. Dilute with 50 ml of water, add 2 ml of nitric acid (~1000 g/l) TS and then titrate with the ammonium thiocyanate solution to the first appearance of a red-brown colour, using 2 ml of ferric ammonium sulfate (45 g/l) TS as indicator.

Ammonium thiocyanate (10 g/l) TS. A solution of ammonium thiocyanate R containing 10 g of NH_4SCN per litre.

Ammonium thiocyanate (75 g/l) TS. A solution of ammonium thiocyanate R containing about 75 g/l of NH_4SCN (approximately 1 mol/l).

Ammonium thiocyanate R. NH_4SCN (SRIP, 1963, p. 40).

Ammonium thiocyanate/cobalt(II) nitrate TS

Procedure. Dissolve 20 g of ammonium thiocyanate R and 5 g of cobalt(II) nitrate R in 100 ml of water. Add sufficient sodium chloride R to saturate the solution.

Ammonium vanadate R. NH_4VO_3.

Description. A white to slightly yellowish, crystalline powder.

Solubility. Slightly soluble in water; soluble in ammonia (~100 g/l) TS.

Amodiaquine hydrochloride RS. International Chemical Reference Substance.

Amoxicillin trihydrate RS. International Chemical Reference Substance.

Amphotericin B RS. International Chemical Reference Substance (containing the declared content of tetraenes).

Ampicillin RS. International Chemical Reference Substance.

Ampicillin sodium RS. International Chemical Reference Substance.

Ampicillin trihydrate RS. International Chemical Reference Substance.

Amyl alcohol R. $C_5H_{12}O$ (SRIP, 1963, p. 42).

Anhydrotetracycline hydrochloride RS. International Chemical Reference Substance.

Aniline (25 g/l) TS. A solution of aniline R containing about 25 g of C_6H_7N per litre.

Aniline R. C_6H_7N (SRIP, 1963, p. 43).

Anisaldehyde R. 4-Methoxybenzaldehyde; $C_8H_8O_2$.
Description. A colourless to pale yellow, oily liquid.
Solubility. Very slightly soluble in water, miscible with ethanol (~750 g/l) TS and ether R.
Mass density. ρ_{20} = about 1.125 kg/l.
Boiling point. About 248 °C.
Anisaldehyde TS
Procedure. Mix in the following order 0.5 ml of anisaldehyde R, 10 ml of glacial acetic acid R, 85 ml of methanol R, and 5 ml of sulfuric acid (~1760 g/l) TS.

Anisaldehyde/methanol TS
Procedure. Slowly add 10 ml of glacial acetic acid R and 5 ml of sulfuric acid (~1760 g/l) TS to 55 ml of methanol R, and cool to room temperature. Separately add 0.5 ml of anisaldehyde R to 30 ml of methanol R. Mix the two solutions thoroughly.
Storage. Keep anisaldehyde/methanol TS protected from light.
Note: Anisaldehyde/methanol TS should be freshly prepared.

Anisaldehyde/sulfuric acid TS
Procedure. Add 5 ml of anisaldehyde R to 10 ml of sulfuric acid (~1760 g/l) TS.

Anthrone R. $C_{14}H_{10}O$.
Description. A pale yellow, crystalline powder.
Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and in sulfuric acid (~100 g/l) TS.

Solubility in carbon tetrachloride R. Add 0.5 g to 10 ml of carbon tetrachloride R; a clear, non-fluorescent solution is produced.
Melting range. 154–156 °C.

Anthrone TS
Procedure. Dissolve 35 mg of anthrone R in 100 ml of sulfuric acid (~1760 g/l) TS.

Anthrone TS2
Procedure. Dissolve 200 mg of anthrone R in 100 ml of sulfuric acid (~1760 g/l) TS.

Antimony sodium tartrate (50 g/l) TS. A solution of antimony sodium tartrate R containing about 50 g/l of $C_4H_4NaO_7Sb$.

Antimony sodium tartrate R. $C_4H_4NaO_7Sb$.
Description. Hygroscopic, transparent or whitish scales or powder.
Solubility. Soluble in 1.5 parts of water; practically insoluble in ethanol (~710 g/l) TS.

Antimony trichloride R. $SbCl_3$. Contains not less than 97.0% of $SbCl_3$.
Description. Colourless crystals.
Solubility. Very soluble in dehydrated ethanol R and in chloroform R (may form a slightly turbid solution).
Chloroform-insoluble substances. Dissolve 5.0 g in 25 ml of chloroform R, filter through a tared filtering crucible, wash the crucible with several portions of chloroform R, and dry at 105 °C; it leaves a residue of not more than 1.0 mg.
Assay. Dissolve 0.5 g, accurately weighed, in 30 ml of water containing 4.0 g of potassium sodium tartrate R, add 2 g of sodium hydrogen carbonate R, and titrate with iodine (0.1 mol/l) VS. Each ml of iodine (0.1 mol/l) VS is equivalent to 11.41 mg of $SbCl_3$.
Note: In moist air fumes may be evolved.

Antimony trichloride TS
Procedure. Dissolve 22 g of antimony trichloride R in 100 ml of ethanol-free chloroform R, add 2.5 ml of acetyl chloride R, and allow to stand for 30 minutes.

Aprotinin R. A polypeptide consisting of a chain of 58 amino acids. A commercially available reagent of suitable grade.

Arachis oil R. Use arachis oil as described in the monograph for "Arachis oil".

Argon R. Ar. Contains not less than 99.995% of Ar.

Carbon monoxide. When used as described in the test for carbon monoxide in medicinal gases, after passage of 10 litres of argon at a flow rate of 4 litres per hour, not more than 0.05 ml of sodium thiosulfate (0.002 mol/l) VS is required for the titration (0.6 µl/l).

Arsenic trioxide R. As_2O_3 (SRIP, 1963, p. 44).

Arsenic trioxide R1. Arsenic trioxide R that has been prepared according to either of the following methods:

1. Recrystallize arsenic trioxide R from a boiling mixture of 20 parts of hydrochloric acid (~420 g/l) TS and 5 parts of water. After cooling, collect the crystals and recrystallize them from boiling water until the mother liquor has a pH greater than 4.0. Dry the crystals to constant weight over silica gel, desiccant, R.
2. Sublime arsenic trioxide R in an appropriate apparatus.

pH Value. Heat to boiling for a few minutes 1.0 g in 20 ml of water, filter and cool; the filtrate has a pH greater than 4.0.

Chlorides. Dissolve 10 mg in sufficient water to produce 10 ml. Acidify with 1 drop of nitric acid (~1000 g/l) TS and add 2 drops of silver nitrate (0.1 mol/l) VS; the solution remains clear and colourless for not less than 2 minutes.

Sulfides. To a solution of 5.0 g in a mixture of 10 ml of sodium hydroxide (~80 g/l) TS and 15 ml of water add 2 drops of lead acetate (80 g/l) TS; the solution remains colourless.

Loss on drying. Dry to constant weight over silica gel, desiccant, R; it loses not more than 0.1 mg/g.

Sulfated ash. Not more than 0.1 mg/g.

Arsenic, dilute, AsTS. One millilitre contains 10 µg of arsenic.

Procedure. Dilute 1 ml of strong arsenic AsTS with sufficient water to produce 100 ml.

Note: Dilute arsenic AsTS must be freshly prepared.

Arsenic, strong, AsTS

Procedure. Dissolve 0.132 g of arsenic trioxide R in 6 ml of sodium hydroxide (~80 g/l) TS, by gentle heating. Dilute the cooled solution with 20 ml of water, and add 50 ml of hydrochloric acid (~250 g/l) TS and sufficient water to produce 100 ml.

Artemether RS. International Chemical Reference Substance.

Artemisinin RS. International Chemical Reference Substance.

Artemotil RS. International Chemical Reference Substance.

Artenimol RS. International Chemical Reference Substance.

Artesunate RS. International Chemical Reference Substance.

Atenolol RS. International Chemical Reference Substance.

Atenolol for column validation RS. International Chemical Reference Substance.

Atropine sulfate RS. International Chemical Reference Substance.

Azathioprine RS. International Chemical Reference Substance.

Azo violet R. 4-(4-Nitrophenylazo)resorcinol; Magneson I: $C_{12}H_9N_3O_4$.
Description. A red powder.
Melting temperature. About 193 °C with decomposition.

Azo violet TS
Procedure. Dissolve 0.2 g of azo violet R in a mixture of 1 volume of toluene R and 2 volumes of cyclohexane R.

Bacitracin zinc RS. International Chemical Reference Substance.

Barium chloride (0.5 mol/l) VS. Barium chloride R, dissolved in water to contain 104.2 g of $BaCl_2$ in 1000 ml.
Method of standardization. Ascertain the exact concentration of the 0.5 mol/l solution in the following manner: Place 10.0 ml of sulfuric acid (0.5 mol/l) VS in a flask, dilute with 40 ml of water, add 2 drops of thorin (2 g/l) TS and titrate slowly with the barium chloride solution to a reddish colour.

Barium chloride (50 g/l) TS. A solution of barium chloride R containing about 52 g/l of $BaCl_2$ (approximately 0.25 mol/l).

Barium chloride R. $BaCl_2,2H_2O$ (SRIP, 1963, p. 45).

Barium hydroxide (0.15 mol/l) VS. Barium hydroxide R dissolved in carbon-dioxide-free water R to contain 25.7 g of $Ba(OH)_2$ in 1000 ml.

Barium hydroxide (15 g/l)TS. A solution of barium hydroxide R in carbon-dioxide-free water R containing about 15 g of $Ba(OH)_2$ per litre.
Note: Barium hydroxide (15 g/l) TS must be freshly prepared.

Barium hydroxide R. $Ba(OH)_2,8H_2O$ (SRIP, 1963, p. 46).

Barium nitrate (0.01 mol/l) VS. Barium nitrate R, dissolved in water to contain 2.614 g of $Ba(NO_3)_2$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/l solution in the following manner: Place 10.0 ml of sulfuric acid (0.01 mol/l) VS in a flask, dilute with 40 ml of water, add 2 drops of thorin (2 g/l) TS and 2 drops of methylthioninium chloride (0.2 g/l) TS, and titrate slowly with the barium nitrate solution from yellow to a pink colour.

Barium nitrate R. $Ba(NO_3)_2$ (SRIP, 1963, p. 47).

Barium oxide R. BaO.

Description. White to yellowish-white lumps or powder. Absorbs moisture and carbon dioxide on exposure to air.

Storage. Store in tightly closed containers.

Barium sulfate suspension TS

Procedure. Mix 15 ml of barium chloride (0.5 mol/l) VS with 55 ml of water and 20 ml of sulfate-free ethanol (~750 g/l) TS, add 5 ml of potassium sulfate (174 mg/l) TS, and dilute with sufficient water to produce 100 ml.

Note. Barium sulfate suspension TS must be freshly prepared.

Beclometasone dipropionate RS. International Chemical Reference Substance.

Beef extract R. A residue from beef broth obtained by extracting fresh, sound, lean beef by cooking with water and evaporating the resulting broth at a low temperature, usually under reduced pressure until a thick pasty residue is obtained.

Benzalkonium chloride TS. A mixture of alkylbenzyldimethylammonium chlorides. It contains in 1 litre not less than 470 g and not more than 530 g of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$.

Description. A clear, colourless to pale yellow, syrupy liquid; odour, aromatic.

Miscibility. Miscible with water and ethanol (~750 g/l) TS.

Assay. Dissolve 4 g, accurately weighed, in sufficient water to produce 100 ml. Transfer 25 ml to a separator, add 25 ml of chloroform R, 10 ml of sodium hydroxide (0.1 mol/l) VS, and 10 ml of freshly prepared potassium iodide (50 g/l) TS. Shake well, allow to separate, and run off the chloroform layer. Shake the aqueous solution with 3 further quantities of chloroform R, each of 10 ml, and discard the chloroform solutions. Add 40 ml of hydrochloric acid (~420 g/l) TS, cool, and titrate with potassium iodate (0.05 mol/l) VS until the solution becomes pale brown in colour. Add 2 ml of chloroform R and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of water, 6 ml of potassium iodide (80 g/l) TS, and 40 ml of hydrochloric acid (~420 g/l) TS with potassium iodate (0.05 mol/l) VS in a similar manner; the difference between the titrations represents the amount of potassium iodate (0.05 mol/l) VS required. Each ml of potassium iodate (0.05 mol/l) VS is equivalent to 35.40 mg of $C_{22}H_{40}ClN$. Determine the

mass density using a pycnometer as described in 1.3 Determination of mass density and relative density and calculate in g/l the proportion of $C_{22}H_{40}ClN$.

Note: A solution in water foams strongly when shaken.

Benzalkonium chloride TS1

Procedure. Dilute 2 ml of benzalkonium chloride TS with sufficient water to produce 100 ml.

Benzene R. C_6H_6 (SRIP, 1963, p. 48).

Benznidazole RS. International Chemical Reference Substance.

Benzoic acid R. $C_7H_6O_2$. Contains not less than 99.8% of $C_7H_6O_2$.

Description. Colourless, light, feathery crystals or a white, microcrystalline powder; odour, characteristic, faint.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS, and ether R.

Methanol-insoluble substances. Dissolve 20 g in 200 ml of methanol R and digest under complete reflux for 30 minutes. Filter through a tared filtering crucible, wash thoroughly with methanol R, and dry at 105 °C; it leaves a residue of not more than 1.0 mg.

Assay. Dissolve about 0.5 g, accurately weighed, in 15 ml of ethanol (~750 g/l) TS, previously neutralized to phenol red/ethanol TS, add 20 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, using phenol red/ethanol TS as indicator. Repeat the operation without the substance being examined and make any necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 12.21 mg of $C_7H_6O_2$.

Benzophenone R. Diphenyl ketone; $C_{13}H_{10}O$.

A commercially available reagent of suitable grade.

Melting point. About 49 °C.

Benzoyl chloride R. C_7H_5ClO (SRIP, 1963, p. 50).

Benzoyl peroxide, hydrous R. $C_{14}H_{10}O_4, \times H_2O$.

A commercially available reagent of suitable grade.

Description. A white, amorphous or granular powder.

Note: For safety reasons it should be kept moistened with about 23% w/w water.

Benzyl alcohol R. C_7H_8O.

Description. A colourless liquid; almost odourless.

Miscibility. Miscible with 25 parts of water; miscible with ethanol (~750 g/l) TS, and ether R.

Boiling temperature. About 204 °C.

Mass density. ρ_{20} = about 1.05 kg/l.

Benzyl benzoate R. $C_{14}H_{12}O_2$.
Benzyl benzoate as described in the monograph for "Benzyl benzoate".
A commercially available reagent of suitable grade.
Description. A clear, colourless, oily liquid.

Benzylpenicillin potassium RS. International Chemical Reference Substance.

Benzylpenicillin sodium R. $C_{16}H_{17}N_2NaO_4S$. Contains not less than 96.0% and not more than 102.0% of $C_{16}H_{17}N_2NaO_4S$, calculated with reference to the dried substance.
Description. A white or almost white, crystalline powder; odourless or with a faint characteristic odour.
Solubility. Soluble in about 0.5 part of water; practically insoluble in ether R.

Benzylpenicillin sodium RS. International Chemical Reference Substance.

Benzylpenicillin sodium TS
Procedure. Dissolve 0.03 g of benzylpenicillin sodium R in sufficient phosphate buffer, pH 7.0, TS, to produce 10 ml. This solution contains not less than 3 mg/ml of benzylpenicillin sodium R.

Bephenium hydroxynaphthoate RS. International Chemical Reference Substance.

Betamethasone RS. International Chemical Reference Substance.

Betamethasone sodium phosphate RS. International Chemical Reference Substance.

Betamethasone valerate RS. International Chemical Reference Substance.

Biperiden hydrochloride RS. International Chemical Reference Substance.

Biperiden RS. International Chemical Reference Substance.

4,4′-Bis(dimethylamino)benzophenone R. Tetramethyldiaminobenzophenone; $C_{17}H_{20}N_2O$.
Other name. Michler's ketone.
Melting point. About 176 °C.

9,9′-Bisanthracene-10,10′(9H,9′H)-dione RS. International Chemical Reference Substance.

Bismuth oxynitrate R. Approximately $4BiNO_3(OH)_2,BiO(OH)$ (SRIP, 1963, p. 50).

Bismuth subnitrate R. Bismuth hydroxide nitrate oxide; $Bi_5O(OH)_9(NO_3)_4$. Bismuth subnitrate is a basic salt, the composition of which varies with the conditions under which it is prepared. It contains not less than 71.5% and not more than 74.5% of Bi, calculated with reference to the dried substance.

Description. A white powder.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS; soluble in hydrochloric acid (~250 g/l) TS and nitric acid (~1000 g/l) TS.

Blue tetrazolium R. $C_{40}H_{32}Cl_2N_8O_2$. 3,3′-Dianisole-bis-[4,4′-(3,5-diphenyl) tetrazolium chloride].

Description. Lemon-yellow crystals.

Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS, and methanol R; practically insoluble in acetone R and ether R.

Molar absorptivity. Its molar absorptivity in methanol R at 252 nm, is not less than 60 000.

Suitability test. Prepare the following standard solution: Dissolve in ethanol (~750 g/l) TS a suitable quantity of hydrocortisone R, previously dried at 105 °C for 3 hours and accurately weighed, and prepare by a step-by-step dilution a solution containing about 30 µg/ml. Transfer 10-, 15- and 20-ml quantities of the standard solution to separate glass-stoppered 50-ml conical flasks. Add 10 ml and 5 ml, respectively, of ethanol (~750 g/l) TS to the flasks containing the 10- and 15-ml quantities of the standard solution and swirl to mix. To each of the flasks, and to a fourth flask, representing the blank, containing 20 ml of ethanol (~750 g/l) TS, add 2.0 ml of a solution prepared by dissolving 0.05 g of the blue tetrazolium R in 10 ml of ethanol (~750 g/l) TS, mix, and then add 2.0 ml of tetramethylammonium hydroxide/ethanol TS. Mix, allow the flasks to stand in the dark for 90 minutes, and determine the absorbances at 525 nm against the blank. Plot the absorbances on the abscissa and the amount of hydrocortisone on the ordinate scale of arithmetic coordinate paper, and draw the curve of best fit; the absorbance of each solution is proportional to the concentration and the absorbance of the solution containing 200 µg of hydrocortisone is not less than 0.50.

Blue tetrazolium/ethanol TS

Procedure. Dissolve 0.5 g of blue tetrazolium R in sufficient aldehyde-free ethanol (~750 g/l) TS, warming slightly if necessary, to produce 100 ml.

Blue tetrazolium/sodium hydroxide TS

Procedure. Immediately before use, mix 1 volume of a 2 mg/ml solution of blue tetrazolium R in water with 3 volumes of a 0.12 g/ml solution of sodium hydroxide R in methanol TS.

Borate buffer, pH 8.0, TS

Procedure. Dissolve 0.25 g of boric acid R and 0.30 g of potassium chloride R in 50 ml of carbon-dioxide-free water R, add 3.97 ml of carbonate-free sodium

hydroxide (0.2 mol/l) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 ml.

Borate buffer, pH 9.0, TS

Procedure. Dissolve 1.24 g of boric acid R in about 100 ml of water, add 8.3 ml of sodium hydroxide (1 mol/l) VS, and add sufficient water to produce 200 ml.

Borate buffer, pH 9.6, TS

Procedure. Dissolve 0.25 g of boric acid R and 0.30 g of potassium chloride R in 50 ml of carbon-dioxide-free water R, add 36.85 ml of carbonate-free sodium hydroxide (0.2 mol/l) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 ml.

Boric acid (50 g/l) TS. A solution of boric acid R containing about 50 g/l of H_3BO_3.

Boric acid R. H_3BO_3. Contains not less than 99.0% of H_3BO_3.

Description. White crystals or scales of a somewhat pearly lustre or a white, crystalline powder.

Solubility. Soluble in 20 parts of water, in 3 parts of boiling water, and in 16 parts of ethanol (~750 g/l) TS.

Water-insoluble substances. 1.0 g dissolves in 30 parts of water; the solution is clear and colourless.

Ethanol-insoluble substances. 1.0 g dissolves in 10 ml of boiling ethanol (~750 g/l) TS; the solution is not more than faintly turbid.

Assay. Dissolve about 1 g, accurately weighed, in 30 ml of water; add 50 ml of glycerol R, previously neutralized to phenolphthalein/ethanol TS, and titrate with carbonate-free sodium hydroxide (1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 61.83 mg of H_3BO_3.

Brilliant green R. [4-[*p*-(Diethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-1-ylidene]diethylammonium hydrogen sulfate; $C_{27}H_{34}N_2O_4S$; C.I. 42040; Malachite green G; C.I. Basic green 1.

Description. Small, glistening, golden crystals.

Brilliant green/acetic acid TS

Procedure. Dissolve 0.5 g of brilliant green R in sufficient glacial acetic acid R1 to produce 100 ml.

Bromine AsTS

Procedure. Dissolve 30 g of potassium bromide R in 40 ml of water, add 30 g of bromine R and dilute with sufficient water to produce 100 ml. The solution complies with the following test: Evaporate 10 ml nearly to dryness on a water-bath, add 50 ml of water, 10 ml of hydrochloric acid (~250 g/l) AsTS,

and sufficient stannous chloride AsTS to reduce the remaining bromine, and apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 1-ml standard stain, showing that the amount of arsenic does not exceed 1 µg/ml.

Bromine R. Br_2 (SRIP, 1963, p. 51).

Bromine TS1. A saturated solution of bromine R.

Bromocresol green R. $C_{21}H_{14}Br_4O_5S$ (SRIP, 1963, p. 52).

Bromocresol green TS1

Procedure. Dissolve 0.05 g of bromocresol green R and 1.021 g of potassium hydrogen phthalate R in 6 ml of sodium hydroxide (0.2 mol/l) VS, and dilute to 100 ml with water. Filter if necessary.

Bromocresol green/ethanol TS

Procedure. Warm 0.1 g of bromocresol green R with 2.9 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~710 g/l) TS; after solution has been effected, add a sufficient quantity of ethanol (~150 g/l) TS to produce 250 ml.

Bromocresol purple R. $C_{21}H_{16}Br_2O_5S$ (SRIP, 1963, p. 52).

Bromocresol purple/ethanol TS

Procedure. Dissolve 0.05 g of bromocresol purple R in 100 ml of ethanol (~750 g/l) TS, and filter if necessary.

Bromophenol blue (1 g/l) TS. A solution of bromophenol blue R containing about 1.0 g of $C_{19}H_{10}Br_4O_5S$ per litre.

Bromophenol blue R. $C_{19}H_{10}Br_4O_5S$ (SRIP, 1963, p. 52).

Bromophenol blue TS

Procedure. Dissolve 0.05 g of bromophenol blue R with gentle heating in 3.73 ml of sodium hydroxide (0.02 mol/l) VS and dilute to 100 ml with water.

Bromophenol blue/ethanol TS

Procedure. Warm 0.1 g of bromophenol blue R with 3.2 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~710 g/l) TS; after solution has been effected, add a sufficient quantity of ethanol (~150 g/l) TS to produce 250 ml.

Bromothymol blue R. $C_{27}H_{28}Br_2O_5S$ (SRIP, 1963, p. 53).

Bromothymol blue/dimethylformamide TS
Procedure. Dissolve 1.0 g of bromothymol blue R in sufficient dimethylformamide R to produce 100 ml.

Bromothymol blue/ethanol TS
Procedure. Warm 0.1 g of bromothymol blue R with 3.2 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~710 g/l) TS; after solution has been effected, add a sufficient quantity of ethanol (~150 g/l) TS to produce 250 ml.

Brown stock standard TS
Procedure. To 35.0 ml of cobalt colour TS, add 17.0 ml of copper colour TS, 8.0 ml of dichromate colour TS, dilute to 100.0 ml with iron colour TS, and mix.

Bupivacaine hydrochloride RS. International Chemical Reference Substance.

2-Butanol R. $C_4H_{10}O$ (SRIP, 1963, p. 53).

1-Butanol R. [*n*-Butanol R]. $C_4H_{10}O$ (SRIP, 1963, p. 54).
tert.-**Butanol R.** 2-Methylpropan-2-ol; $(CH_3)_3COH$.
Description. A colourless liquid or solid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS, and ether R.
Boiling range. Not less than 95% distils between 81 and 83 °C.
Melting range. 24–26 °C.
Mass density. ρ_{20} = 0.778–0.782 kg/l.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.05 mg/ml.

Butyl acetate R. $C_6H_{12}O_2$.
Description. A clear, colourless, flammable liquid; odour, characteristic.
Miscibility. Slightly miscible with water; miscible with ethanol (~750 g/l) TS.
Mass density. ρ_{20} = about 0.88 kg/l.

tert-Butyl methyl ether R. 1,1-Dimethylethyl methyl ether; $C_5H_{12}O$.
A commercially available reagent of suitable grade.
Description. A clear, colourless liquid; inflammable.
Refractive index. n_D^{20} = 1.3756.
Relative density. d_4^{20} = 0.740–0.742.

1-Butylamine R. 1-Aminobutane; $C_4H_{11}N$.
Description. A colourless to pale yellow inflammable liquid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS, and ether R.
Boiling range. Not less than 95% distils between 76 and 78 °C.
Mass density. ρ_{20} = about 0.740 kg/l.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, using about 5 ml of the liquid; not more than 10 mg/ml.
Acid impurities. To 50 ml add 5 drops of azo violet TS, and titrate quickly with sodium methoxide (0.1 mol/l) VS to a deep blue endpoint, taking precautions to prevent absorption of atmospheric carbon dioxide, e.g. by use of an atmosphere of nitrogen; not more than 1.0 ml of sodium methoxide (0.1 mol/l) VS is required for neutralization.

Butylated hydroxyanisole R. Use $C_{11}H_{16}O_2$.
Butylated hydroxyanisole as described in the monograph in "Butylated hydroxyanisole".

Butylated hydroxytoluene R. 2,6-Di-*tert*-butyl-4-methylphenol, $C_{15}H_{24}O$.
Description. Colourless crystals, or a white, crystalline powder.
Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS; very soluble in ether R.
Melting temperature. About 70 °C.
Sulfated ash. Not more than 1.0 mg/g.

Cadmium acetate R. $(CH_3CO_2)_2Cd,2H_2O$. Contains not less than 98.0% of $(CH_3CO_2)_2Cd,2H_2O$.
Description. Colourless crystals.
Solubility. Soluble in water.
Assay. Dissolve 1 g, accurately weighed, in 50 ml of water, add 25 ml of ammonia (~260 g/l) TS, and titrate with disodium edetate (0.1 mol/l) VS, using methylthymol blue mixture R as indicator, until the blue solution becomes colourless or grey. Each ml of disodium edetate (0.1 mol/l) VS is equivalent to 26.65 mg of $(CH_3CO_2)_2Cd,2H_2O$.

Caesium chloride R. CsCl.
A commercially available reagent of suitable grade.

Caffeine RS. International Chemical Reference Substance.

Calcium acetate (0.25 mol/l) VS. Calcium acetate R, dissolved in water to contain 44.04 g of $Ca(C_2H_3O_2)_2,H_2O$ in 1000 ml.

Calcium acetate R. $Ca(C_2H_3O_2)_2,H_2O$ (SRIP, 1963, p. 56).

Calcium carbonate R1. $CaCO_3$ (SRIP, 1963, p. 56).

Calcium carbonate R2. $CaCO_3$. Calcium carbonate R1 of suitable quality to serve as a primary standard for the standardization of disodium edetate.

Calcium chloride (3.7g/l) TS. A solution of anhydrous calcum chloride R containing about 9 g of $CaCl_2$ per litre.

Calcium chloride (55 g/l) TS. A solution of hydrated calcium chloride R containing about 55 g/l of $CaCl_2$ (approximately 0.5 mol/l).

Calcium chloride, anhydrous, R. [calcium chloride R]. $CaCl_2$ (SRIP, 1963, p. 58).

Calcium chloride, hydrated, R. $CaCl_2,6H_2O$ (SRIP, 1963, p. 58).

Calcium fluoride R. CaF_2.
Description. A white powder.
Solubility. Practically insoluble in water; slightly soluble in dilute acids.

Calcium folinate RS. International Chemical Reference Substance.

Calcium hydroxide R. $Ca(OH)_2$ (SRIP, 1963, p. 59).

Calcium hydroxide TS
Procedure. Prepare a saturated solution of calcium hydroxide R.
Note: Calcium hydroxide TS must be freshly prepared.

Calcium standard (10 µg/ml Ca) TS
Procedure. Dissolve 2.50 g of dried calcium carbonate R2 in 15 ml of acetic acid (~300 g/l) TS and dilute with water to 1000 ml (solution A). Dilute 10.0 ml of this solution with water to produce 1000 ml.

Calcium standard (100 µg/ml Ca), ethanolic, TS
Procedure. Dilute 100.0 ml of solution A, described under calcium standard (10 µg/ml Ca) TS, with sufficient ethanol (~750 g/l) TS to produce 1000 ml.

Calcium sulfate R. $CaSO_4,2H_2O$ (SRIP, 1963, p. 62).

Calcium sulfate TS
Procedure. Shake 5 g of calcium sulfate hemihydrate R for 1 hour with 100 ml of water and filter.

Calcium sulfate, hemihydrate R. Plaster of Paris, $CaSO_4, \frac{1}{2} H_2O$.
Description. A white powder which, when mixed with half its weight of water, rapidly solidifies to a hard and porous mass.

Calcon carboxylic acid indicator mixture R
Procedure. Mix 0.1 g of calcon carboxylic acid R with 10 g of anhydrous sodium sulfate R.

Calcon carboxylic acid R. 2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthyl-azo)-3-naphthoic acid; $C_{21}H_{14}N_2O_7S,3H_2O$.
Description. A dark-brown powder with a violet tint.

Solubility. Practically insoluble in water; slightly soluble in methanol R and in ethanol (~750 g/l) TS; freely soluble in solutions of alkali hydroxides.

Calcon indicator mixture R
Procedure. Mix 0.1 g of calcon R with 10 g of anhydrous sodium sulfate R.

Calcon R. Monosodium salt of 2-hydroxy-1-[(2-hydroxy-1-naphthyl)-azo]naphthalene-4-sulfonic acid; C.I. Mordant Black 17, C.I. No. 15705, Eriochrome Blue Black R, Solochrome Dark Blue; $C_{20}H_{13}N_2NaO_5S$.

Captopril disulfide RS. International Chemical Reference Substance.

Captopril RS. International Chemical Reference Substance.

Carbamazepine RS. International Chemical Reference Substance.

Carbidopa RS. International Chemical Reference Substance.

Carbomer R. Carbomer suitable for thin-layer chromatography. A high relative molecular mass cross-linked polymer of acrylic acid; it contains a large proportion (56–68%) of carboxylic acid (-COOH) groups after drying at 80 °C for 1 hour.
pH value. The pH of a 10 g/l suspension is about 3.
Viscosity. Whilst stirring continuously, prepare a suspension containing 2.5 g in 500 ml of water. Maintain at 25 ± 0.2 °C for 30 minutes, then add 0.2 ml of phenolphthalein/ethanol TS, 1 ml of bromothymol blue/ethanol TS and, whilst stirring, neutralize using a mixture of equal volumes of sodium hydroxide (~400 g/l) TS and water until a uniform blue colour is obtained (check the pH which must be 7.3–7.8). The dynamic viscosity of the neutralized preparation is 30–40 Pa s (300–400 poise).

Carbon dioxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for hydrazine and crystal violet indicators. The minimum value indicated is 100 µl/l or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Carbon dioxide R. CO_2.
Description. A colourless gas; odourless.
Solubility. Soluble in about 1.3 parts by volume of water.

Carbon disulfide IR. Carbon disulfide R that complies with the following test: The infrared absorption spectrum of a 1.0-mm layer, as described in method 4 under 1.7 Spectrophotometry in the infrared region, and examined over the range 4000–670 cm^{-1} shows an absorbance of less than 0.1 in

the regions 4000–3030 cm^{-1}, 2635–2440 cm^{-1}, 2000–1755 cm^{-1}, and 1265–935 cm^{-1}, and an absorbance of less than 0.17 in the region 800–715 cm^{-1}.

Carbon disulfide R. CS_2 (SRIP, 1963, p. 62).

Carbon monoxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for di-iodine pentoxide, selenium dioxide and fuming sulfuric acid indicators. The minimum value indicated is 5 µl/l or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Carbon monoxide R. CO.
A commercially available gas of suitable grade.

Carbon tetrachloride R. CCl_4 (SRIP, 1963, p. 63).

Carboxymethylcellulose R. A suitable grade for column chromatography.

Cellulose R1. Microcrystalline cellulose suitable for thin-layer chromatography.
Description. A fine, white homogeneous powder.
Particle size. Less than 30 µm.
Note: A suspension of about 25 g of cellulose R1 in 90 ml of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cellulose R2. Cellulose suitable for thin-layer chromatography.
Description. A fine, white homogeneous powder.
Particle size. Less than 30 µm.
Note: A suspension of about 15 of cellulose R2 in 100 ml of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cellulose R3. Cellulose suitable for thin-layer chromatography.
Description. A fine, white homogeneous powder.
Composition. Cellulose (particle size less than 30 µm) containing a fluorescent indicator having an optimal intensity at 254 nm.
Note: A suspension of about 25 g of cellulose R3 in 100 ml of water is used in the preparation of the coating for thin-layer chromatographic plates.

Cephaëline hydrochloride R. $C_{28}H_{38}N_2O_4,2HCl,7H_2O$.
Description. A white, crystalline powder.
Specific optical rotation. Use a 20 mg/ml solution; $[\alpha]_D^{20\,°C} = +25°$.

Cephalin TS
Procedure. Place a quantity between 0.5 and 1.0 g of acetone-dried ox brain R into a centrifuge tube, add 20 ml of acetone R, and allow to stand for 2 hours. Centrifuge for 2 minutes and decant the supernatant liquid. Dry the residue

under reduced pressure, add to it 20 ml of chloroform R, and allow to stand for 2 hours, shaking frequently. Separate the solid material by filtration or centrifugation and evaporate the chloroform under reduced pressure. Suspend the residue in 5–10 ml of sodium chloride (9 g/l) TS. Solvents used to prepare cephalin TS should contain a suitable antioxidant, for example, a solution of 0.02 g/l of butylated hydroxyanisole R.

Storage. Store in a freezer or keep in a freeze-dried state.

Note: The reagent must be used within 3 months.

Ceric ammonium nitrate (0.01 mol/l) VS

Procedure. Dissolve 5.482 g of ceric ammonium nitrate R in sufficient nitric acid (1 mol/l) VS to produce 1000 ml, and filter.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/l solution in the following manner: Measure accurately 2.0 ml of freshly standardized ferrous ammonium sulfate (0.1 mol/l) VS into a flask and dilute with water to about 100 ml. Add 1 drop of nitrophenanthroline TS, and titrate with the ceric ammonium nitrate solution to a colourless end-point. From the volume of ferrous ammonium sulfate (0.1 mol/l) VS taken and the volume of ceric ammonium nitrate solution consumed, calculate the molarity.

Ceric ammonium nitrate R. $Ce(NO_3)_4,2NH_4NO_3$.

Description. Small orange-red monoclinic crystals.

Solubility. Very soluble in water.

Insoluble matter. To 5 g, accurately weighed, add 10 ml of sulfuric acid (~1760 g/l) TS, stir, and cautiously add 90 ml of water to dissolve. Heat to boiling and digest in a covered beaker on a water-bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105 °C. The weight of the residue does not exceed 2.5 mg.

Assay. Dissolve 2.5 g, accurately weighed and previously dried at 85 °C for 24 hours, in 10 ml of sulfuric acid (~190 g/l) TS and add 40 ml of water. Add a few drops of *o*-phenanthroline TS and titrate with ferrous sulfate (0.1 mol/l) VS. Each ml of ferrous sulfate (0.1 mol/l) VS is equivalent to 54.8 mg of $Ce(NO_3)_4,2NH_4NO_3$.

Ceric ammonium sulfate (0.1 mol/l) VS

Procedure. Dissolve 65.0 g of ceric ammonium sulfate R in a mixture of 500 ml of water and 30 ml of sulfuric acid (~1760 g/l) TS. Allow to cool and dilute to 1000 ml with water.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Accurately weigh about 0.2 g of arsenic trioxide R1 and dissolve by gently heating in 15 ml of sodium hydroxide (0.2 mol/l) VS. Add to the clear solution 50 ml of sulfuric acid (~100 g/l) TS, 0.15 ml of a 2.5 mg/ml solution of osmium tetroxide R in sulfuric acid (~100 g/l) TS, and 0.1 ml of *o*-phenanthroline TS. Titrate the solution with

the ceric ammonium sulfate solution until the red colour disappears. Titrate slowly as the end-point is approached.

Ceric ammonium sulfate R. Ammonium cerium (IV) sulfate dihydrate, $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$. Contains not less than 95.0% of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

Description. Yellow crystals or an orange-yellow, crystalline powder.

Solubility. Slowly soluble in water; insoluble in ethanol (~750 g/l) TS.

Assay. Dissolve about 1 g, accurately weighed, in 50 ml of sulfuric acid (~100 g/l) TS, add 0.1 ml of a 10 mg/ml solution of osmium tetroxide R, and titrate with sodium arsenite (0.05 mol/l) VS, using *o*-phenanthroline TS as indicator. Each ml of sodium arsenite (0.05 mol/l) VS is equivalent to 63.26 mg of $Ce(SO_4)_2,2(NH_4)_2SO_4,2H_2O$.

Ceric ammonium sulfate/nitric acid TS

Procedure. Dissolve 5 g of ceric ammonium sulfate R in sufficient nitric acid (~130 g/l) TS to produce 100 ml.

Ceric amonium nitrate TS

Procedure. Dissolve 6.25 g of ceric ammonium nitrate R in 10 ml of nitric acid (15 g/l) TS.

Shelf-life. Use within 3 days of preparation.

Ceric sulfate (0.1 mol/l) VS

Procedure. Dissolve ceric sulfate R, equivalent to 33.23 g of $Ce(SO_4)$, in a mixture of 28 ml of sulfuric acid (~1760 g/l) TS and 500 ml of water, dilute to 1000 ml and mix. Allow the solution to stand for 48 hours and filter through a sintered glass filter.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Place about 25 ml, accurately measured, in a glass-stoppered flask, dilute with 80 ml of water, add 10 ml of phosphoric acid (~105 g/l) TS and 2.5 g of potassium iodide R, and allow the solution to stand for 15 minutes. Add 1 g of sodium carbonate R and titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator.

Ceric sulfate (35 g/l) TS. A solution of ceric sulfate R containing about 33 g/l of $Ce(SO_4)_2$.

Ceric sulfate R. Usually $Ce(SO_4)_2,4H_2O$ (SRIP, 1963, p. 63).

Charcoal R. (SRIP, 1963, p. 64).

Chloralose R. $C_8H_{11}Cl_3O_6$.

Description. A colourless, crystalline powder.

Melting temperature. About 187 °C.

Specific optical rotation. Use a 50 mg/ml solution in ethanol (~750 g/l) TS; $[\alpha]_D^{20\,°C} = +19\,°$.

Chloramphenicol disodium disuccinate RS. International Chemical Reference Substance.

Chloramphenicol palmitate RS. International Chemical Reference Substance.

Chloramphenicol sodium succinate RS. International Chemical Reference Substance.

Chloramphenicol RS. International Chemical Reference Substance.

Chloraniline R. 4-Chloroaniline, C_6H_6ClN.
Description. White or faintly coloured crystals.
Melting temperature. About 70 °C.

Chloride standard (5 µg/l) TS
Procedure. Weigh accurately 82.4 mg of sodium chloride R and dissolve in sufficient water to produce 100 ml. Dilute 1.0 ml of this solution with water to 100 ml.

Chlorine R. Cl_2 (SRIP, 1963, p. 65).

Chlorine TS. A saturated solution of chlorine R in water.
Note: Chlorine TS must be freshly prepared.

7-Chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl) quinoline-3-carboxylic acid RS (ciprofloxacin by-compound A). International Chemical Reference Substance.

1-Chloro-2,4 dinitrobenzene R. $C_6H_3ClN_2O_4$.
A commercially available reagent of suitable grade.
Melting point. About 144 °C.

1-Chloro-2,4 dinitrobenzene/ethanol TS
Procedure. Weigh 5 g of 1-Chloro-2,4 dinitrobenzene R and dissolve in sufficient ethanol (~750 g/l) TS to produce 100 ml.

5-Chloro-2-methylaminobenzophenone RS. International Chemical Reference Substance.

2-(4-Chloro-3-sulfamoyl)benzoic acid RS. International Chemical Reference Substance.

2-Chloro-4-nitroaniline R. $C_6H_5ClN_2O_2$.
Description. A yellow to brown, crystalline powder.
Solubility. Slightly soluble in water; soluble in ethanol (~750 g/l) TS.
Melting range. 106–108 °C.
Sulfated ash. Not more than 0.5 mg/g.

4-Chloroacetanilide R. C_8H_8ClNO.
Description. Colourless, needle-shaped crystals or a white to pale yellow, crystalline powder.
Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS and ether R.
Melting temperature. About 180 °C.

Chloroform R. $CHCl_3$ (SRIP, 1963, p. 66).

Chloroform, ethanol-free, R
Procedure. Shake 20 ml of chloroform R gently but thoroughly with 20 ml of water for 3 minutes, draw of the chloroform layer, and wash twice more with 20-ml quantities of water. Finally filter the chloroform through a dry filter-paper, shake it well with 5 g of powdered anhydrous sodium sulfate R for 5 minutes, allow the mixture to stand for 2 hours, and decant or filter the clear chloroform.

Chloroquine sulfate RS. International Chemical Reference Substance.

Chlorphenamine hydrogen maleate RS. International Chemical Reference Substance.

Chlorpromazine hydrochloride RS. International Chemical Reference Substance.

Chlortalidone RS. International Chemical Reference Substance.

Chlortetracycline hydrochloride RS. International Chemical Reference Substance.

Chromic acid TS
Procedure. Dissolve 84 g of chromium trioxide R in 700 ml of water and add slowly while stirring 400 ml of sulfuric acid (~1760 g/l) TS.

Chromium trioxide R. CrO_3 (SRIP, 1963, p. 68).

Ciclosporin RS. International Chemical Reference Substance.

Ciclosporin U RS. International Chemical Reference Substance.

Cimetidine RS. International Chemical Reference Substance.

Cinchonidine R. $C_{19}H_{22}N_2O$.
Description. A white, crystalline powder.
Solubility. Soluble in ethanol (~750 g/l) TS.
Melting temperature. About 207 °C.
Specific optical rotation. Use a 50 mg/ml solution in ethanol (~750 g/l) TS; $[\alpha]_D^{20\,°C}$ = −105 to −110 °.

Cinchonine R. $C_{19}H_{22}N_2O$ (SRIP, 1963, p. 69).

Ciprofloxacin hydrochloride RS. International Chemical Reference Substance.

Ciprofloxacin RS. International Chemical Reference Substance.

Cisplatin RS. International Chemical Reference Substance.

Citrate buffer, pH 4.0, TS
Procedure. Dissolve 10.5 g of citric acid R in about 100 ml of water, add 100 ml of sodium hydroxide (1 mol/l) VS, and dilute to 500 ml with water. Dilute 100 ml of hydrochloric acid (0.1 mol/l) VS with the solution prepared above to produce 250 ml.

Citrate buffer, pH 5.4, TS
Procedure. Dissolve 2.101 g of citric acid R in water, add 20 ml of sodium hydroxide (1 mol/l) VS, and dilute with sufficient water to produce 100 ml. Mix 76.5 ml of this solution with 23.5 ml of sodium hydroxide (0.1 mol/l) VS.

Citric acid (180 g/l) FeTS. A solution of citric acid FeR containing about 183 g/l of $C_6H_8O_7$.

Citric acid (20 g/l) TS. A solution of citric acid R containing about 20 g of $C_6H_8O_7$ per litre.

Citric acid FeR. Citric acid R that complies with the following test: Dissolve 0.5 g of citric acid R in 40 ml of water, add 2 drops of mercaptoacetic acid R, mix, make alkaline with ammonia (~100 g/l) FeTS and dilute to 50 ml with water; no pink colour is produced.

Citric acid PbR. Citric acid R free of lead.

Citric acid R. $C_6H_8O_7,H_2O$ (SRIP, 1963, p. 69).

Citric acid, copper-free, R. Citric acid R, that complies with the following additional test: Dissolve 0.50 g in 20 ml of water, make alkaline with ammonia (~100 g/l) TS, dilute to 50 ml with water, and add 1 ml of sodium diethyldithiocarbamate (0.8 g/l) TS; no yellow colour is produced.

Clindamycin hydrochloride RS. International Chemical Reference Substance.

Clindamycin phosphate RS. International Chemical Reference Substance.

Clofazimine RS. International Chemical Reference Substance.

Clomifene citrate RS. International Chemical Reference Substance.

Clomifene citrate Z-isomer RS. See Zuclomifene RS.

Cloxacillin sodium RS. International Chemical Reference Substance.

Cobalt colour TS. A solution containing 60.0 g/l of $CoCl_2,6H_2O$.

Procedure. Prepare a solution containing 6.000 g of $CoCl_2,6H_2O$ in 100 ml by diluting the strong cobalt colour TS with sulfuric acid (~10 g/l) TS, as necessary.

Cobalt colour, strong, TS

Procedure. Dissolve 8.0 g of cobaltous chloride R in 120 ml of sulfuric acid (~10 g/l) TS, filter the solution, if necessary, and determine the concentration of $CoCl_2,6H_2O$.

Assay. Dilute 5.0 ml with sufficient water to produce 100 ml. Transfer 10.0 ml of this solution to a glass-stoppered flask, add 10 ml of water, 0.5 ml of hydrogen peroxide (~60 g/l) TS and 10 ml of sodium hydroxide (~80 g/l) TS. Add a few boiling chips to the flask, and boil the contents of the flask until the excess of hydrogen peroxide is completely decomposed (approximately 10 minutes). Cool the flask, add 20 ml of water, 1 g of potassium iodide R, and 25 ml of hydrochloric acid (2 mol/l) VS. Close the flask with the stopper and allow to stand until the precipitate dissolves. Titrate the liberated iodine with sodium thiosulfate (0.01 mol/l) VS using starch TS as indicator. Each ml of sodium thiosulfate (0.01 mol/l) VS is equivalent to 2.380 mg of $CoCl_2,6H_2O$.

Cobalt(II) chloride (30 g/l) TS. A solution of cobalt(II) chloride R containing about 30 g of $CoCl_2$ per litre.

Cobalt(II) chloride (5 g/l) TS. A solution of cobalt(II) chloride R containing about 5 g of $CoCl_2$ per litre.

Cobalt(II) chloride R. Cobaltous chloride; $CoCl_2,6H_2O$ (SRIP, 1963, p. 70).

Cobalt(II) chloride TS
Procedure. Dissolve 6.5 g of cobalt(II) chloride R in a sufficient quantity of a mixture of 2.5 ml of hydrochloric acid (~250 g/l) TS and 97.5 ml of water to produce 100 ml.

Cobalt(II) nitrate (10 g/l) TS
Procedure. Dissolve about 1.6 g of cobalt(II) nitrate R in sufficient water to produce 100 ml.

Cobalt(II) nitrate (100 g/l) TS. A solution of cobalt(II) nitrate R containing about 100 g of $Co(NO_3)_2$ per litre.

Cobalt(II) nitrate R. $Co(NO_3)_2,6H_2O$.
Description. Small red crystals.
Solubility. Very soluble in water.

Cobaltous chloride R. $CoCl_2,6H_2O$ (SRIP, 1963, p. 70).

Cobaltous chloride TS
Procedure. Dissolve 6.5 g of cobaltous chloride R in a sufficient quantity of a mixture of 2.5 ml of hydrochloric acid (~250 g/l) TS and 97.5 ml of water to produce 100 ml.

Codeine R. $C_{18}H_{21}NO_3,H_2O$.
Description. Colourless crystals or a white, crystalline powder; odourless.
Solubility. Slightly soluble in water; freely soluble in ethanol (~750 g/l) TS and ether R.
Melting temperature. About 156 °C.
Specific optical rotation. Use a 20 mg/ml solution in ethanol (~750 g/l) TS; $[\alpha]_D^{20\,°C}$ = −142 to − 146°.

Colbatous thiocyanate TS
Procedure. Dissolve 6.8 g of cobaltous chloride R and 4.3 g of ammonium thiocyanate R in sufficient water to produce 100 ml.

Colchicine RS. International Chemical Reference Substance.

Colecalciferol RS. International Chemical Reference Substance.

Congo red paper R. (SRIP, 1963, p. 72).

Copper colour TS. A solution containing 60.0 g/l of $CuSO_4,5H_2O$.
Procedure. Prepare a solution containing 6.000 g of $CuSO_4,5H_2O$ in 100 ml by diluting the strong copper colour TS with sulfuric acid (~10 g/l) TS, as necessary.

Copper colour, strong, TS

Procedure. Dissolve 8.0 g of copper(II) sulfate R in 120 ml of sulfuric acid (~10 g/l) TS, filter the solution, if necessary, and determine the concentration of $CuSO_4,5H_2O$.

Assay. Dilute 5.0 ml with sufficient water to produce 100 ml. Transfer 10.0 ml of this solution to a glass-stoppered flask, add 20 ml of water, 1 g of potassium iodide R, and 5 ml of glacial acetic acid R. After 10 minutes, titrate the liberated iodine with sodium thiosulfate (0.01 mol/l) VS, using starch TS as indicator. Each ml of sodium thiosulfate (0.01 mol/l) VS is equivalent to 2.497 mg of $CuSO_4,5H_2O$.

Copper edetate TS

Procedure. To 2 ml of a 20 mg/ml solution of copper(II) acetate R, add 2 ml of disodium edetate (0.1 mol/l) VS, and dilute to 50 ml with water.

Copper standard (10 µg/ml Cu) TS

Procedure. Dissolve 0.393 g of copper(II) sulfate R in sufficient water to produce 100 ml and dilute 10.0 ml of this solution to produce 1000 ml.

Copper standard TS1

Procedure. Dissolve 1.965 g of copper(II) sulfate R, accurately weighed, in sufficient hydrochloric acid (0.1 mol/l) VS to produce 1000 ml.

Copper standard TS2

Procedure. Transfer 3.0 ml of copper standard TS1 to a 1000-ml flask and dilute with hydrochloric acid (0.1 mol/l) VS to produce 1000 ml. This solution contains 1.5 µg of Cu per ml.

Copper tetramine hydroxide TS

Procedure. Dissolve 34.5 g of copper(II) sulfate R in 100 ml of water. Stir and add, drop by drop, ammonia (~260 g/l) TS until the precipitate formed has completely dissolved. Keep the temperature below 20 °C and add slowly, while stirring, 30 ml of sodium hydroxide (~400 g/l) TS. Filter the precipitate through a sintered glass filter (porosity 16–40 µm), and wash with water until the filtrate is clear. Add 200 ml of ammonia (~260 g/l) TS to the precipitate, stir, and filter.

Copper(II) acetate (45 g/l) TS. A solution of copper(II) acetate R containing about 50 g of $C_4H_6CuO_4,H_2O$ per litre.

Copper(II) acetate R. $C_4H_6CuO_4,H_2O$. Contains not less than 98.0% of $C_4H_6CuO_4,H_2O$.

Description. Blue-green crystals or powder; odour, resembling that of acetic acid.

Solubility. Soluble in water.

Assay. Dissolve 0.8 g, accurately weighed, in 50 ml of water, add 2 ml of acetic acid (~300 g/l) TS and 3 g of potassium iodide R, and titrate the liberated

iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator, until only a faint blue colour remains; add 2 g of potassium thiocyanate R and continue the titration until the blue colour disappears. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 19.97 mg of $C_4H_6CuO_4,H_2O$.

Copper(II) chloride R. $CuCl_2,2H_2O$.
Description. Bluish green, deliquescent crystals.
Solubility. Freely soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.

Copper(II) chloride/ammonia TS
Procedure. Dissolve 22.5 g of copper(II) chloride R in 200 ml of water and add 100 ml of ammonia (~260 g/l) TS.

Copper(II) sulfate (1 g/l) TS. A solution of copper(II) sulfate R containing 1 g of $CuSO_4$ per litre.

Copper(II) sulfate (160 g/l) TS. A solution of copper(II) sulfate R containing about 160 g/l of $CuSO_4$.

Copper(II) sulfate (80 g/l) TS. A solution of copper(II) sulfate R containing about 80 g of $CuSO_4$ per litre (approximately 0.5 mol/l).

Copper(II) sulfate R. $CuSO_4,5H_2O$ (SRIP, 1963, p. 72).

Copper(II) sulfate/ammonia TS
Procedure. Dissolve 50 g of copper(II) sulfate R in 1000 ml of ammonia (~35 g/l) TS.

Copper(II) sulfate/pyridine TS
Procedure. Dissolve 4 g of copper(II) sulfate R in 90 ml of water and add 30 ml of pyridine R.
Note: Copper(II) sulfate/pyridine TS must be freshly prepared.

o-Cresol R. 2-Methylphenol; C_7H_8O.
Description. Colourless to pale brownish-yellow crystals or liquid; odour, resembling that of phenol.
Miscibility. Miscible with ethanol (~750 g/l) TS, and ether R; miscible with about 50 parts of water.
Mass density. ρ_{20} = about 1.05 kg/l.
Refractive index. n_D^{20} = 1.540 – 1.550.
Boiling temperature. About 190 °C.
Freezing temperature. Not below 30.5 °C.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 1.0 mg/ml.

Storage. Store in a tightly closed container, protected from light and oxygen.
Note: On exposure to light and air *o*-cresol R darkens in colour.

Cresol red R. $C_{21}H_{18}O_5S$.
Description. A red-brown powder.
Solubility. Slightly soluble in water; soluble in ethanol (~750 g/l) TS and in dilute solutions of alkali hydroxides.

Cresol red/ethanol TS
Procedure. Warm 0.05 g of cresol red R with 2.65 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~710 g/l) TS; after solution has been effected, add sufficient ethanol (~150 g/l) TS to produce 250 ml.

Crystal violet R. $C_{25}H_{30}ClN_3$ (SRIP, 1963, p. 73).

Crystal violet/acetic acid TS. A solution of crystal violet R dissolved in glacial acetic acid R1 containing about 5 g/l.

Culture medium Cm1
Procedure. Dissolve 6.0 g of dried peptone R, 4.0 g of pancreatic digest of casein R, 3.0 g of water-soluble yeast extract R, 1.5 g of beef extract R, 1.0 g of glucose hydrate R, and 10–20 g of agar R in sufficient water to produce 1000 ml.
Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm2
Procedure. Dissolve 17.0 g of pancreatic digest of casein R, 3.0 g of papaic digest of soybean meal R, 5.0 g of sodium chloride R, 2.5 g of dipotassium hydrogen phosphate R, 2.5 g of glucose hydrate R, and 10–20 g of agar R in about 500 ml of water. Heat the solution, add 10.0 g of polysorbate 80 R and dilute immediately with a sufficient amount of water to produce 1000 ml.
Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm3
Procedure. Dissolve 9.4 g of dried peptone R, 4.7 g of water-soluble yeast extract R, 2.4 g of beef extract R, 10.0 g of sodium chloride R, 10.0 g of glucose hydrate R, and 15–25 g of agar R in sufficient water to produce 1000 ml.
Note: The quantity of agar R used should permit the culture medium to be of adequate firmness to support cylinders or to permit holes to be cut without tearing the gel layer.

Culture medium Cm4. Fluid mercaptoacetate (thioglycolate) medium.

Procedure. Thoroughly grind in a mortar in the following order: 0.5 g of L-cystine R, 2.5 g of sodium chloride R, 5.5 g of glucose hydrate R, 0.75 g of agar R, 5.0 g of water-soluble yeast extract R and 15.0 g of pancreatic digest of casein R. Add a small volume of hot water, transfer to a suitable container, and add sufficient water to produce 1000 ml. Complete the solution by heating in a boiling water-bath, taking care to ensure complete solution of the L-cystine R. Add 0.3 ml of mercaptoacetic acid R or 0.5 g of sodium mercaptoacetate R (the latter is preferred as a more stable compound) and sufficient sodium hydroxide (1 mol/l) VS so that the pH of the final and sterilized medium will be 7.0–7.2. Reheat the solution, but do not boil, filter, if necessary, through cotton wool and add 1.0 ml of resazurin sodium (1 g/l) TS. Distribute the solution into suitable vessels and sterilize by autoclaving for 18–20 minutes at 121 °C and cool promptly to 25 °C.

Storage. At 20–30 °C, avoiding excessive light.

Note: If the uppermost portion of the medium has changed to a pink colour and this exceeds one-fifth of the depth of the medium, it is unsuitable for use, but may be restored once by heating in steam.

Culture medium Cm5. Soybean-casein digest medium.

Procedure. Dissolve in water 17.0 g of pancreatic digest of case in R, 3.0 g of papaic digest of soybean meal R, 5.0 g of sodium chloride R, 2.5 g of dipotassium hydrogen phosphate R, and 2.5 g of glucose hydrate R. Warm the solution slightly, then cool it to room temperature and add sufficient water to produce 1000 ml. Adjust the pH of the solution with sodium hydroxide (1 mol/l) VS, if necessary, so that the pH of the final and sterilized medium will be 7.1–7.5. Filter, if necessary to clarify, distribute the solution into suitable vessels and sterilize in an autoclave for 18–20 minutes at 121 °C.

Culture medium Cm6. Fluid mercaptoacetate (thioglycolate) medium with penicillinase.

Procedure. Use culture medium Cm4 with sufficient sterile penicillinase TS added to inactivate the penicillin activity of the test material. Add penicillinase TS to individual vessels containing sterile culture medium Cm4 using an aseptic technique. Prior to use, or at the time of the test, incubate a representative number of the vessels with culture medium Cm6 at 30–32 °C for 24–48 hours and examine for sterility.

Culture medium Cm7. Soybean-casein digest medium with penicillinase.

Procedure. Use culture medium Cm5 to which 5.0 ml of polysorbate 80 R has been added before sterilization and with sufficient sterile penicillinase TS added to inactivate the penicillin activity of the test material. Add penicillinase TS to individual vessels containing sterile culture medium Cm5, using an aseptic technique.

Culture medium Cm8

Procedure. Dissolve 10 g of dried peptone R, 10 g of beef extract R, 10 g of glycerol R, 3.0 g of sodium chloride R, and 17 g of agar R in sufficient water to produce 1000 ml. Adjust the pH with sodium hydroxide (0.05 mol/l) VS to 6.9–7.1, and sterilize in an autoclave at 121 °C for 18–20 minutes.

Culture medium Cm9

Procedure. Dissolve 10 g of dried peptone R, 10 g of beef extract R, 10 g of glycerol R, and 3.0 g of sodium chloride R in sufficient water to produce 1000 ml. Adjust the pH with sodium hydroxide (0.05 mol/l) VS to 6.9–7.1, and sterilize in an autoclave at 121 °C for 18–20 minutes.

Culture medium Cm10

Procedure. Dissolve 1 g of water-soluble yeast extract R, 5 g of ammonium nitrate R, 5 g of sodium dihydrogen phosphate R, 5 g of anhydrous glucose R, and 14 g of agar R in sufficient water to produce 1000 ml.

Culture medium Cm11

Procedure. Dissolve 10 g of dried peptone R, 6 g of beef extract R, and 15 g of agar R in sufficient water to produce 1000 ml. Adjust the pH of the solution with sodium hydroxide (1 mol/l) VS, if necessary, so that the pH of the final and sterilized medium will be 7.8. Filter, if necessary to clarify, distribute the solution into suitable vessels and sterilize in an autoclave at 121 °C for 18–20 minutes.

Cyanide/oxalate/thiosulfate TS

Procedure. To 2.0 ml of ammonia (~100 g/l) TS, add in the following order: 1.5 ml of ammonium oxalate (50 g/l) TS, 15 ml of potassium cyanide (50 g/l) TS, 45 ml of sodium acetate (60 g/l) TS, 120 ml of sodium thiosulfate (320 g/l) TS, 75 ml of sodium acetate (60 g/l) TS, and 35 ml of hydrochloric acid (1 mol/l) VS.

Note: Cyanide/oxalate/thiosulfate TS must be prepared immediately before use.

Cyanoethylmethyl silicone gum R. A suitable grade to be used in gas-liquid chromatography.

Cyanogen bromide TS

Caution. Very toxic; avoid inhalation of vapours.

Procedure. Add drop by drop, while cooling, potassium cyanide (100 g/l) TS to bromine TS1 until the colour disappears.

Note: Cyanogen bromide TS must be prepared immediately before use.

Cyclohexane R. C_6H_{12} (SRIP, 1963, p. 74).

Cyclohexane R1. Cyclohexane R showing a fluorescence that is not more intense than that of a solution of 2 µg/ml of quinine R in sulfuric acid

(0.05 mol/l) VS when measured at 460 nm using a 1-cm layer and an excitation beam at 365 nm.

1-Cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid RS (ciprofloxacin desfluoro-compound). International Chemical Reference Substance.

L-Cystine R. $C_6H_{12}N_2O_4S_2$ (SRIP, 1963, p. 75).

Cytarabine RS. International Chemical Reference Substance.

Dacarbazine RS. International Chemical Reference Substance.

Dacarbazine related compound A RS. International Chemical Reference Substance.

Dacarbazine related compound B RS. International Chemical Reference Substance.

Dactinomycin RS. International Chemical Reference Substance.

Dantron R. 1,8-dihydroxyanthraquinone; $C_{14}H_8O_4$.
A commercially available reagent of suitable grade.
Description. An orange, microcrystalline powder.
Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and ether R.
Melting point. About 193 °C.

Dapsone RS. International Chemical Reference Substance.

Dexamethasone acetate RS. International Chemical Reference Substance.

Dexamethasone RS. International Chemical Reference Substance.

Dexamethasone sodium phosphate RS. International Chemical Reference Substance.

Dextromethorphan hydrobromide RS. International Chemical Reference Substance.

1,4-Di[2-(4-methyl-5-phenyloxazole)]benzene R.

3,3′-Diaminobenzidine tetrahydrochloride R. $C_{12}H_{14}N_4,4HCl,2H_2O$.
A commercially available reagent of suitable grade.
Description. An almost white or slightly pink powder.
Dimethyl-POPOP $C_{26}H_{20}N_2O_2$. Suitable for scintillation counting.

3,3′-Diaminobenzidine tetrahydrochloride (5g/l) TS. A solution of 3,3′-diaminobenzidine tetrahydrochloride R containing 5g of $C_{12}H_{14}N_4,4HCl$ per litre.

Diammonium hydrogen phosphate (100g/l) TS. A solution of diammonium hydrogen phosphate R containing about 100g of $(NH_4)_2HPO_4$ per litre.

Diammonium hydrogen phosphate R [ammonium phosphate R]. $(NH_4)_2HPO_4$ (SRIP, 1963, p.38).

Diatomaceous support R

Description. White granules of silica consisting chiefly of the skeletons of diatoms. The material is flux-calcined to sequester coloured metallic oxides in a colourless form, and is supplied commercially in various forms, such as: acid-washed, silanized, and alkali-washed.

Diazepam RS. International Chemical Reference Substance.

Diazobenzenesulfonic acid TS

Procedure. To 0.9g of sulfanilic acid R add 10ml of hydrochloric acid (~250g/l) TS and sufficient water to produce 100ml. To 3ml of this solution add 5ml of sodium nitrite (3g/l) TS, cool in ice for 5 minutes, add a further 20ml of sodium nitrite (3g/l) TS, and again cool in ice; dilute with water to 100ml, keeping the solution cold.

Note: Diazobenzenesulfonic acid TS must be freshly prepared and should not be used until at least 15 minutes after its preparation.

Diazomethane TS

Caution. Diazomethane is explosive in the gaseous state and its explosive decomposition is easily initiated by rough surfaces. Do not use apparatus with ground-glass joints or boiling stones of any kind. Diazomethane is highly toxic and all operations should be conducted under a well-ventilated hood.

Procedure. Prepare a solution of 0.4g of potassium hydroxide R in 10ml of ethanol (~750g/l) TS and add it to a solution of 2.14g of *N*-methyl-*N*-nitrosotoluene-4-sulfonamide R in 30ml of ether R while cooling in ice. If a precipitate forms, add just sufficient ethanol (~750g/l) TS to dissolve it. After 5 minutes, gently distil the ethereal diazomethane solution from a water-bath. Diazomethane TS contains about 10g of CH_2N_2 per litre.

Alternative procedures. Other procedures for the evolution of diazomethane, using other starting materials, may also be employed, provided the resulting solution has the required concentration of CH_2N_2.

Diazoxide RS. International Chemical Reference Substance.

Dibromomethane R. Methylene bromide, CH_2Br_2.
Description. Colourless to yellowish liquid.
Miscibility. Miscible with ethanol (~750 g/l) TS, ether R, and acetone R.

Dibutyl ether R. Di-*n*-butyl ether, $C_8H_{18}O$.
Caution. Dibutyl ether R tends to form explosive peroxides, especially when anhydrous.
Description. A colourless liquid.
Miscibility. Practically immiscible with water; miscible with ethanol (~750 g/l) TS and ether R.
Boiling range. 140–143 °C.
Mass density. $\rho_{20} = 0.769$ kg/l.
Refractive index. $n_D^{20} = 1.344$.

Dibutyl phthalate R. Di-*n*-butyl phthalate, $C_{16}H_{22}O_4$.
Description. A clear, colourless or faintly coloured liquid.
Miscibility. Very slightly miscible with water; miscible with ethanol (~750 g/l) TS and ether R.
Mass density. $\rho_{20} = 1.043 - 1.048$ kg/l.
Refractive index. $n_D^{20} = 1.492 - 1.495$.
Sulfated ash. Not more than 0.2 mg/ml.

Dichloroethane R. 1,2-Dichloroethane, $C_2H_4Cl_2$ (SRIP, 1963, p. 76).

Dichlorofluorescein R. $C_{20}H_{10}Cl_2O_5$.
Description. A light orange-coloured, crystalline powder.
Solubility. Sparingly soluble in water; soluble in ethanol (~750 g/l) TS.

Dichlorofluorescein TS
Procedure. Dissolve 0.2 g of dichlorofluorescein R in 100 ml of methanol R.

Dichloromethane R. Methylene chloride, CH_2Cl_2.
Description. A clear colourless, mobile liquid.
Miscibility. Freely miscible with ethanol (~750 g/l) TS and ether R.
Boiling range. Not less than 95% distils between 39 and 41 °C.
Residue on evaporation. Leaves, after evaporation on a water-bath and drying at 105 °C, not more than 0.5 mg/ml.

2,6-Dichloroquinone chlorimide R. $C_6H_2Cl_3NO$ (SRIP, 1963, p. 77).

2,6-Dichloroquinone chlorimide/ethanol TS
Procedure. Dissolve 0.5 g of 2,6-dichloroquinone chlorimide R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Dichromate colour TS. A solution containing 4.904 g/l of $K_2Cr_2O_7$.

Procedure. Prepare a solution containing 490.35 mg of $K_2Cr_2O_7$ in 100 ml by diluting the strong dichromate colour TS with sulfuric acid (~10 g/l) TS, as necessary.

Dichromate colour, strong, TS

Procedure. Dissolve 6.0 g of potassium dichromate R in 120 ml of sulfuric acid (~10 g/l) TS, filter the solution, if necessary, and determine the concentration of $K_2Cr_2O_7$.

Assay. Dilute 5.0 ml with sufficient water to produce 50 ml. Transfer 10.0 ml of this solution to a glass-stoppered flask, add 10 ml of water, 2 g of potassium bicarbonate R, and 20 ml of sulfuric acid (~100 g/l) TS. Loosely close the flask with its stopper. When the gas evolution has ceased, add 1 g of potassium iodide R, keep the flask for 5 minutes in a dark place and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 4.904 mg of $K_2Cr_2O_7$.

Dicloxacillin sodium RS. International Chemical Reference Substance.

Dicoumarol RS. International Chemical Reference Substance.

Didanosine RS. International Chemical Reference Substance.

Didanosine for system suitability RS. International Chemical Reference Substance.

Diethoxytetrahydrofuran R. $C_8H_{16}O_3$. Mixture of the cis and trans isomers.

Description. Colourless or slightly yellow, clear liquid.

Miscibility. Practically immiscible with water; miscible with ethanol (~750 g/l) TS and ether R.

Mass density. ρ_{20} = about 0.975 kg/l.

Refractive index. n_D^{20} = 1.418.

Diethoxytetrahydrofuran/acetic acid TS

Procedure. Mix 1 ml of diethoxytetrahydrofuran R with sufficient glacial acetic acid R to produce 100 ml.

Diethyl phthalate R. $C_{12}H_{14}O_4$.

Mass density. ρ_{20} = about 1.117 kg/l.

Refractive index. n_D^{20} = 1.500 – 1.505.

Diethylamine R. $C_4H_{11}N$. Contains not less than 99.5% of $C_4H_{11}N$.

Description. A clear, colourless liquid.

Mass density. ρ_{20} = 0.702 – 0.704 kg/l.

Refractive index. n_D^{20} = 1.384 – 1.386.

Assay. Add about 3 g, accurately weighed, to 50 ml of sulfuric acid (0.5 mol/l) VS and titrate the excess acid with sodium hydroxide (1 mol/l) VS, using methyl red/ethanol TS as indicator. Each ml of sulfuric acid (0.5 mol/l) VS is equivalent to 73.14 mg of $C_4H_{11}N$.

Diethylaminoethylcellulose R. A suitable grade for column chromatography.

Diethylcarbamazine dihydrogen citrate RS. International Chemical Reference Substance.

Diethylene glycol R. $C_4H_{10}O_3$.
Description. A colourless to faintly yellow liquid having a mild odour.
Miscibility. Freely miscible with water, ethanol (~750 g/l) TS, ether R and acetone R.
Boiling range. Between 240 and 250 °C.
Mass density (ρ_{20}). 1.117 – 1.120 kg/l.
Acidity. Transfer 60 g to a 250-ml conical flask, add phenolphthalein/ethanol TS and titrate with potassium hydroxide/ethanol (0.02 mol/l) VS, to a pink colour that remains stable for at least 15 seconds. Not more than 2.5 ml should be consumed.

Diethylene glycol succinate R. A suitable grade to be used in gas-liquid chromatography.

Diethylphenylenediamine sulfate TS
Procedure. To 250 ml of water add about 2 ml of sulfuric acid (~1760 g/l) TS and 50 ml of disodium edetate (0.01 mol/l) VS. Dissolve 1.1 g of *N,N*-diethyl-*p*-phenylenediamine sulfate R into this solution and dilute with sufficient water to produce 1000 ml.

N,N-Diethyl-p-phenylenediamine sulfate R. *N,N*-Diethyl-1,4-phenylenediamine sulfate; $C_{10}H_{16}N_2,H_2SO_4$.
A commercially available reagent of suitable grade.
Description. A white or slightly coloured powder.
Melting point. About 185 °C, with decomposition.
Storage. *N,N*-Diethyl-*p*-phenylenediamine sulfate R should be kept protected from light.

Diethyltoluamide RS. International Chemical Reference Substance.

Digitonin R. $C_{55}H_{90}O_{29}$ (SRIP, 1963, p. 78).

Digitonin TS
Procedure. Dissolve 0.10 g of digitonin R in sufficient ethanol (~710 g/l) TS to produce 10 ml.
Note: Digitonin TS must be freshly prepared.

Digitoxin RS. International Chemical Reference Substance.

Digoxin RS. International Chemical Reference Substance.

Diisopropyl ether R. Isopropyl ether; $C_6H_{14}O$.
Description. A colourless liquid; odour, characteristic.
Boiling point. About 68 °C.
Note. Diisopropyl ether is highly flammable.

Diloxanide furoate RS. International Chemical Reference Substance.

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate RS. International Chemical Reference Substance.

Dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate RS. International Chemical Reference Substance.

Dimethyl sulfoxide R. C_2H_6OS.
Description. A colourless liquid; odourless or with a slight, but unpleasant odour.
Mass density. ρ_{20} = 1.10 kg/l.

Dimethylacetamide R. C_4H_9NO.
Description. A colourless liquid.
Boiling temperature. About 166 °C.
Mass density. ρ_{20} = 0.94 kg/l.

Dimethylamine R. C_2H_7N.
Description. A low-boiling liquid, 7 °C; odour, characteristic.
Solubility. Soluble in water, ethanol (~750 g/l) TS, and ether R.

Dimethylamine/ethanol TS. A solution of dimethylamine R in ethanol (~750 g/l) TS containing about 330 g/l of C_2H_7N.
Assay. Dilute 2 ml to 10 ml with ethanol (~750 g/l) TS. Transfer 2 ml to a flask containing 50.0 ml of sulfuric acid (0.05 mol/l) VS and mix. Titrate the excess acid with sodium hydroxide (0.1 mol/l) VS, using methyl red/ethanol TS as indicator. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 4.508 mg of C_2H_7N.

4-Dimethylaminobenzaldehyde R [dimethylaminobenzaldehyde R]. $C_9H_{11}NO$ (SRIP, 1963, p. 78).

4-Dimethylaminobenzaldehyde TS1
Procedure. Dissolve 0.125 g of 4-dimethylaminobenzaldehyde R in a cooled mixture of 65 ml of sulfuric acid (~1760 g/l) TS and 35 ml of water and add 0.2 ml of ferric chloride (25 g/l) TS.
Note: 4-Dimethylaminobenzaldehyde TS1 must be freshly prepared.

4-Dimethylaminobenzaldehyde TS2

Procedure. Dissolve 0.80 g of 4-dimethylaminobenzaldehyde R in a cooled mixture of 80 g of ethanol (~750 g/l) TS and 20 g of sulfuric acid (~1760 g/l) TS.

4-Dimethylaminobenzaldehyde TS3

Procedure. Dissolve 0.5 g of 4-dimethylaminobenzaldehyde R in 50 ml of ethanol (~750 g/l) TS, add 1 ml of hydrochloric acid (~420 g/l) TS, and dilute with sufficient ethanol (~750 g/l) TS to produce 100 ml.

4-Dimethylaminobenzaldehyde TS4

Procedure. Dissolve 2 g of 4-dimethylaminobenzaldehyde R in a mixture of 5 ml of hydrochloric acid (~420 g/l) TS and 95 ml of glacial acetic acid R.

4-Dimethylaminobenzaldehyde TS5

Procedure. Dissolve without heating 2 g of 4-dimethylaminobenzaldehyde R in a mixture of 45 ml of water and 55 ml of hydrochloric acid (~420 g/l) TS.

4-Dimethylaminobenzaldehyde TS6

Procedure. Dissolve 0.2 g of 4-dimethylaminobenzaldehyde R in 20 ml of ethanol (~750 g/l) TS and add 0.5 ml of hydrochloric acid (~420 g/l) TS. Shake the solution with charcoal R and filter. The colour of this test solution is less intense than that of iodine (0.0001 mol/l) VS.

Note: 4-Dimethylaminobenzaldehyde TS6 must be freshly prepared.

4-Dimethylaminocinnamaldehyde R. $C_{11}H_{13}NO$.

Description. Orange crystals, or a crystalline powder; odour, characteristic.

Solubility. Practically insoluble in water; freely soluble in hydrochloric acid (~70 g/l) TS; sparingly soluble in ethanol (~750 g/l) TS and ether R.

4-Dimethylaminocinnamaldehyde TS1

Procedure. Dissolve 2 g of 4-dimethylaminocinnamaldehyde R in a mixture of 100 ml of hydrochloric acid (5 mol/l) VS and 100 ml of ethanol (~750 g/l) TS.

Storage. Store the solution at a temperature of about 0 °C.

4-Dimethylaminocinnamaldehyde TS2

Procedure. Dilute 20 ml of 4-dimethylaminocinnamaldehyde TS1 with sufficient ethanol (~750 g/l) TS to produce 100 ml.

Note: 4-Dimethylaminocinnamaldehyde TS2 must be freshly prepared.

N,N-Dimethylaniline R. $C_8H_{11}N$.

Description. A colourless liquid darkening on storage.

Miscibility. Practically immiscible with water, miscible with ethanol (~750 g/l) TS, and ether R.

Boiling point. About 193 °C.

Mass density. ρ_{20} = 0.96 kg/l.

Dimethylformamide R. C_3H_7NO.
Description. A clear and colourless liquid, having a characteristic odour.
Miscibility. Miscible with water and ethanol (~750 g/l) TS.
Boiling range. Not less than 25% distils at between 152 and 156 °C.
Mass density(ρ_{20}). 0.945–0.947 kg/l.
Acidity and alkalinity. Dissolve 1 g in 10 ml of water, add 2 drops of phenolphthalein/ethanol TS; not more than 0.2 ml of carbonate-free sodium hydroxide (0.01 mol/l) VS is required to produce a red colour. Add 0.3 ml of hydrochloric acid (0.01 mol/l) VS and 5 drops of methyl, red/ethanol TS; an orange colour is produced.

N,N-Dimethyloctylamine R. Octyldimethylamine; $C_{10}H_{23}N$.
A commercially available reagent of suitable grade.
Description. A colourless liquid.
Boiling point. About 195 °C.

Dinitrobenzene R. $C_6H_4N_2O_4$ (SRIP, 1963, p. 79).

Dinitrobenzene/ethanol TS
Procedure. Dissolve 1 g of dinitrobenzene R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

2,4-Dinitrochlorobenzene R. $C_6H_4ClN_2O_4$ (SRIP, 1963, p. 80).

Dinitrogen oxide R. N_2O.
A commercially available gas of suitable grade.

Dinonyl phthalate R. $C_{26}H_{42}O_4$.
Description. A colourless to pale yellow, viscous liquid.
Mass density. $\rho_{20} = 0.97 - 0.98$ kg/l.
Refractive index. $n_D^{20} = 1.482 - 1.489$.
Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, using about 2 ml of the liquid; not more than 1.0 mg/ml.
Acidity. Shake 5.0 g with 25 ml of water for 1 minute. Allow to stand, filter the aqueous layer and add to it 5 drops of phenolphthalein/ethanol TS; not more than 0.3 ml of carbonate-free sodium hydroxide (0.1 mol/l) VS is required for the neutralization (0.5 mg/g expressed as phthalic acid).

Dioxan R. 1,4-Dioxane, $C_4H_8O_2$.
Caution. It is dangerous to determine the boiling range or the residue on evaporation before complying with the test for peroxides.
Description. A clear, colourless liquid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS and ether R.
Boiling range. Not less than 95% distils at between 101 and 105 °C.
Melting temperature. Solidifies when cooled in ice and does not completely remelt at temperatures below 10 °C.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.1 mg/ml.
Mass density(ρ_{20}). About 1.031 kg/l.
Water. Determined by the Karl Fischer method, not more than 5.0 mg/ml.
Peroxide. Add 5 ml to a mixture of 1 g of potassium iodide R dissolved in 10 ml of water, 5 ml of hydrochloric acid (~70 g/l) TS, and 2 ml of starch TS, and mix; not more than a faint blue or brown colour is produced.

Diphenoxylate hydrochloride RS. International Chemical Reference Substance.

Diphenyl ether R. Phenyl ether, $C_{12}H_{10}O$.
Description. A colourless liquid.
Miscibility. Immiscible with water; freely miscible with glacial acetic acid R and with most organic solvents.
Boiling temperature. About 259 °C.
Melting range. 26–28 °C.

Diphenylamine R. $C_{12}H_{11}N$ (SRIP, 1963, p. 81).

Diphenylamine/sulfuric acid TS
Procedure. Dissolve 1.0 g of diphenylamine R in 100 ml of sulfuric acid (~1760 g/l) TS.
Storage. Diphenylamine/sulfuric acid TS must be colourless and should be kept protected from light.

Diphenylbenzidine R. $C_{24}H_{20}N_2$.
Description. A white, or faintly grey-coloured, crystalline powder.
Solubility. Insoluble in water; slightly soluble in ethanol (~750 g/l) TS and acetone R.
Melting range. 246–250 °C.
Sulfated ash. Not more than 1.0 mg/g.
Nitrates. Dissolve 8 mg in a cooled mixture of nitrogen-free sulfuric acid (~1760 g/l) TS and 5 ml of water; the solution is colourless or not more than very pale blue.

1,5-Diphenylcarbazide R. $C_{13}H_{14}N_4O$.
Description. A white, crystalline powder, gradually turning pink on exposure to air.
Melting point. About 174 °C.

Diphenylcarbazide TS
Procedure. Dissolve 0.2 g of 1,5-diphenylcarbazide R in a mixture of 10 ml of glacial acetic acid R and 90 ml of ethanol (~710 g/l) TS.
Sensitivity test to chromate. Dilute 0.5 ml of potassium dichromate (0.0167 mol/l) VS with water to 1000 ml. Dilute 5 ml of this solution to 50 ml with water,

add 0.2 ml of hydrochloric acid (2 mol/l) VS and 0.5 ml of diphenylcarbazide TS; a reddish violet colour is produced.

Diphenylcarbazone R. $C_{13}H_{12}N_4O$ (SRIP, 1963, p. 81).

Diphenylcarbazone/ethanol TS. A solution of diphenylcarbazone R dissolved in ethanol (~750 g/l) TS containing about 1 g/l of $C_{13}H_{12}N_4O$.

1,2-Diphenylethylammonium 3-mercapto-2-methylpropanoate RS. International Chemical Reference Substance.

2,5-Diphenyloxazole R. PPO, $C_{15}H_{11}NO$. Suitable for scintillation counting.

Dipotassium hydrogen phosphate R. K_2HPO_4 (SRIP, 1963, p. 81).

Disodium chromotropate (10 g/l) TS. A solution of disodium chromotropate R containing about 9.5 g of $C_{10}H_6Na_2O_8S_2$ per litre.

Disodium chromotropate R [chromotropic acid sodium salt R]. $C_{10}H_6Na_2O_8S_2,2H_2O$.

Description. A yellow to light-brown powder.

Solubility. Freely soluble in water; insoluble in ethanol (~750 g/l) TS.

Identification. To 0.5 ml of a 2 mg/ml solution add 10 ml of water and 1 drop of ferric chloride (25 g/l) TS; a green colour is produced.

Sensitivity. Dissolve 5 mg in 10 ml of a mixture of 9 ml of sulfuric acid (~1760 g/l) TS and 4 ml of water. Separately dilute 0.5 ml of formaldehyde TS with water to make 1000 ml. Transfer to each of two separate test-tubes 5 ml of the disodium chromotropate solution, to one add 0.2 ml of the formaldehyde solution, and heat both tubes in a water-bath for 30 minutes; a violet colour is produced in the tube containing the formaldehyde solution.

Disodium chromotropate TS

Procedure. Dissolve 5 mg of disodium chromotropate R in 10 ml of a mixture of 9 ml of sulfuric acid (~1760 g/l) TS and 4 ml of water.

Disodium edetate (0.01 mol/l) VS. Disodium edetate R, dissolved in water to contain 3.342 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following an appropriate method, e.g. as described under disodium edetate (0.05 mol/l) VS.

Disodium edetate (0.05 mol/l) VS. Disodium edetate R, dissolved in water to contain 16.71 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 ml.

Method of standardization. Ascertain the exact concentration by an appropriate method. The following method is suitable: Transfer about 200 mg of calcium

carbonate R2, accurately weighed, to a 400-ml beaker, add 10 ml of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 ml of hydrochloric acid (~70 g/l) TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipette, and the watch glass with water, and dilute with water to about 100 ml. While stirring the solution, preferably with a magnetic stirrer, add about 30 ml of the disodium edetate solution from a 50-ml burette. Add 10 ml of sodium hydroxide (~80 g/l) TS and 0.3 g of calcon indicator mixture R or of calcon carboxylic acid indicator mixture R and continue the titration with the disodium edetate solution to a blue end-point. Each 5.005 mg of calcium carbonate is equivalent to 1 ml of disodium edetate (0.05 mol/l) VS.

Disodium edetate (0.1 mol/l) VS. Disodium edetate R, dissolved in water to contain 33.42 g of $C_{10}H_{14}N_2Na_2O_8$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following an appropriate method, e.g. as described under disodium edetate (0.05 mol/l) VS.

Disodium edetate (10 g/l) TS. A solution of disodium edetate R containing about 10 g of $C_{10}H_{14}N_2Na_2O_8$ per litre.

Disodium edetate (20 g/l) TS. A solution of disodium edetate R containing about 20 g of $C_{10}H_{14}N_2Na_2O_8$ per litre.

Disodium edetate (50 g/l) TS. A solution of disodium edetate R containing about 50 g of $C_{10}H_{14}N_2Na_2O_8$ per litre.

Disodium edetate R. $C_{10}H_{14}N_2Na_2O_8,2H_2O$ (SRIP, 1963, p. 82).

Disodium edetate RS. International Chemical Reference Substance.

Disodium hydrogen phosphate (100 g/l) TS. A solution of disodium hydrogen phosphate R containing about 100 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate (28.4 g/l) TS. A solution of anhydrous disodium hydrogen phosphate R containing 28.4 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate (40 g/l) TS. A solution of disodium hydrogen phosphate R containing about 40 g of Na_2HPO_4 per litre.

Disodium hydrogen phosphate R [sodium phosphate R]. $Na_2HPO_4,12H_2O$ (SRIP, 1963, p. 192).

Disodium hydrogen phosphate, anhydrous, R [sodium phosphate, anhydrous, R]. Na_2HPO_4 (SRIP, 1963, p. 193).

Dissolution media. See under 5.5 Dissolution test for solid oral dosage forms.

5,5′-Dithiobis(2-nitrobenzoic acid) R. 3-Carboxy-4-nitrophenyl disulfide; $C_{14}H_8N_2O_8S_2$.
A commercially available reagent of suitable grade.

5,5′-Dithiobis-2-nitrobenzoic acid/methanol TS
Procedure. Dissolve 0.198 g of 5,5′-Dithiobis(2-nitrobenzoic acid) R in sufficient methanol R to produce 500 ml.
Storage. Keep under refrigeration, and warm to room temperature before use.

Dithizone R. $C_{13}H_{12}N_4S$ (SRIP, 1963, p. 83).

Dithizone standard TS
Procedure. Dissolve 10 mg of dithizone R in 1000 ml of chloroform R.
Storage. Store the solution in a glass-stoppered, lead-free bottle, protected from light, and kept at a temperature not exceeding 4 °C.

Dithizone TS
Procedure. Dissolve 0.10 g of dithizone R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Dithranol RS. International Chemical Reference Substance.

Domiphen bromide (10 g/l) TS. A solution of domiphen bromide R containing about 10 g of $C_{22}H_{40}BrNO$ per litre.

Domiphen bromide R. Dodecyldimethyl(2-phenoxyethyl)ammonium bromide; $C_{22}H_{40}BrNO$.
Description. Colourless or faintly yellow, crystalline flakes.
Solubility. Freely soluble in water and ethanol (~750 g/l) TS; soluble in acetone R.
Dopamine hydrochloride RS.
International Chemical Reference Substance.

Doxorubicin hydrochloride RS. International Chemical Reference Substance.

Doxycycline hyclate RS. International Chemical Reference Substance.

Dragendorff reagent TS
Procedure. Shake vigorously to dissolve 0.85 g of bismuth subnitrate R in 10 ml of glacial acetic acid R and 40 ml of water (*solution A*). Dissolve 8 g of potas-

sium iodide R in 20 ml of water (*solution B*). Immediately before use, mix together equal volumes of *solutions A* and *B* and glacial acetic acid R.
Storage. *Solutions A* and *B* must be protected from light.

Dragendorff reagent, modified, TS
Procedure. Add 20 ml of acetic acid (~60 g/l) TS to 4 ml of a mixture of equal volumes of *solutions A* and *B* of Dragendorff reagent TS.
Note: Prepare this solution immediately before use.

Econazole nitrate RS. International Chemical Reference Substance.

Emetine hydrochloride RS. International Chemical Reference Substance.

Endotoxin RS. Second WHO International Standard for Endotoxin as established in 1996, containing 10 000 IU per ampoule, approximately 1 µg of freeze-dried endotoxin from *Escherichia coli*, with 1 mg of PEG and 10 mg of lactose (distributed by the National Institute for Biological Standards and Control (NIBSC), PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QH, England), or another suitable preparation, the activity of which has been determined in relation to the WHO International Standard using the gelation test.

Eosin Y (5 g/l) TS. A solution of eosin Y R containing about 5 g of $C_{20}H_6Br_4Na_2O_5$ per litre.

Eosin YR. Sodium tetrabromofluorescein; $C_{20}H_6Br_4N_2O_5$.
Description. Red to brownish lumps or a powder.
Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Ephedrine sulfate RS. International Chemical Reference Substance.

4-Epianhydrotetracycline hydrochloride RS. International Chemical Reference Substance.

6-Epidoxycycline hydrochloride RS. International Chemical Reference Substance.

Epinephrine hydrogen tartrate R. Epinephrine hydrogen tartrate R as described in the monograph for "Epinephrine hydrogen tartrate", which complies with the following test for the absence of levarterenol:
Levarterenol. Carry out descending 1.14.2 Paper chromatography. Mix 4 volumes of 1-butanol R, 1 volume of glacial acetic acid R, and 5 volumes of water, shake and allow the two layers to separate. Use the lower layer as the stationary phase and the upper layer as the mobile phase. Apply to the paper 20 µl of a solution containing 50 mg/ml of epinephrine hydrogen tartrate R, develop for 5 hours, dry the paper, and spray with a freshly pre-

pared 4.4 mg/ml solution of potassium ferricyanide R dissolved in buffer borate, pH 8.0, TS or another buffer having the same pH may be used; only 1 spot appears, which is pink.

4-Epitetracycline hydrochloride RS. International Chemical Reference Substance.

Ergocalciferol RS. International Chemical Reference Substance.

Ergometrine hydrogen maleate RS. International Chemical Reference Substance.

Ergosterol R. Provitamin D_2; ergosta-5,7,22-trien-3-ol; $C_{28}H_{44}O$. Contains not less than 95.0% of $C_{28}H_{44}O$.
Description. White or almost white needles or a crystalline powder.
Melting temperature. About 163 °C.
Specific optical rotation. Use a 20 mg/ml solution in chloroform R; $[\alpha]_D^{20\,°C} = -133\,°$.

Ergotamine tartrate RS. International Chemical Reference Substance.

Erythromycin ethylsuccinate RS. International Chemical Reference Substance.

Erythromycin lactobionate RS. International Chemical Reference Substance.

Erythromycin RS. International Chemical Reference Substance.

Erythromycin stearate RS. International Chemical Reference Substance.

Estrone RS. International Chemical Reference Substance.

Ethambutol hydrochloride RS. International Chemical Reference Substance.

Ethanol (~150 g/l) TS. A solution of about 210 ml of ethanol (~750 g/l) TS diluted with water to 1000 ml.

Ethanol (~375 g/l) TS. A solution of about 525 ml of ethanol (~750 g/l) TS diluted with water to 1000 ml.

Ethanol (~457 g/l) TS
Procedure. Dilute 519 ml of ethanol (~750 g/l) TS with sufficient water to produce 1000 ml.

Ethanol (~535 g/l) TS
Procedure. Dilute 623 ml of ethanol (~750 g/l) TS with sufficient water to produce 1000 ml.

Ethanol (~600g/l) TS. A solution of about 735ml of ethanol (~750g/l) TS diluted with water to 1000ml.

Ethanol (~675g/l) TS. A solution of about 842ml of ethanol (~750g/l) TS diluted with water to 1000ml.

Ethanol (~710g/l) TS. A solution of about 950ml of ethanol (~750g/l) TS diluted with water to 1000ml.

Ethanol (~750g/l) TS
[ethanol (95 per cent) R] (SRIP, 1963, p. 84).

Ethanol (~750g/l), aldehyde-free TS
[ethanol, aldehyde-free, (95 per cent.) R]. (SRIP, 1963, p. 84).

Ethanol (~750g/l), sulfate-free, TS. Ethanol (~750 g/l) TS that complies with the following test: Evaporate 25ml of ethanol (~750 g/l) TS to a volume of about 2ml, add a mixture of 3ml of hydrochloric acid (~70 g/l) TS and 42ml of water, and 5ml of barium sulfate suspension TS. Proceed as described in 2.2.2 Limit test for sulfates. Sulfate-free ethanol (~750 g/l) TS contains not more than 20µg/ml.

Ethanol, dehydrated, R. C_2H_5OH (SRIP, 1963, p. 85).

Ethanol, neutralized, TS
Procedure. To a suitable quantity of ethanol (~750g/l) TS add 0.5ml of phenolphthalein/ethanol TS and just sufficient carbonate-free sodium hydroxide (0.02mol/l) VS or (0.1mol/l) VS to produce a faint pink colour.
Note: Prepare neutralized ethanol TS just prior to use.

Ether R. $C_4H_{10}O$ (SRIP, 1963, p. 85).

Ether, peroxide-free, R
Procedure. To 1000ml of ether R add 20ml of a solution of 30g of ferrous sulfate R in 55ml of water and shake the mixture with 3ml of sulfuric acid (~1760g/l) TS. Continue shaking until a small sample no longer produces a blue colour when shaken with an equal volume of a 20g/l solution of potassium iodide R and 0.1ml of starch TS.

Ethinylestradiol RS. International Chemical Reference Substance.

Ethionamide RS. International Chemical Reference Substance.

Ethosuximide RS. International Chemical Reference Substance.

Ethyl acetate R. $C_4H_8O_2$ (SRIP, 1963, p. 86).

Ethyl iodide R. C_2H_5I (SRIP, 1963, p. 87).

Ethylene glycol monoethyl ether R. $C_4H_{10}O_2$.
Description. A clear, colourless liquid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS, ether R, and acetone R.
Boiling range. Not less than 95% distils at between 133 and 135 °C.
Mass density(ρ_{20}). About 0.93 kg/l.

Ethylene glycol monomethyl ether R. 2-Methoxyethanol; $C_3H_8O_2$.
Description. A colourless liquid.
Boiling temperature. About 125 °C.
Mass density. ρ_{20} = about 0.96 kg/l.

Ethylene oxide R. C_2H_4O.
A commercially available gas of suitable grade.

Ethylene oxide TS
Procedure. Weigh 1.0 g of cold ethylene oxide stock solution R (equivalent to 2.5 mg of ethylene oxide) into a cold flask containing 40 g of cold macrogol 200 TS. Mix and determine the exact mass, and dilute to a calculated mass to obtain a solution containing 50 µg of ethylene oxide per 1.0 g of solution. Weigh 10.0 g into a flask and dilute with sufficient water to produce 50 ml (10 µg/ml of ethylene oxide). Dilute 10 ml of this solution to 50 ml with water (2 µg/ml of ethylene oxide).
Note: Ethylene oxide TS should be prepared immediately before use.

Ethylene oxide stock solution R
Note: *All operations should be carried out in a fume-hood. The operator must protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask.*
Procedure. Into a dry, clean test-tube, cooled in a mixture of 1 part of sodium chloride R and 3 parts of crushed ice, introduce a slow current of ethylene oxide R gas, allowing condensation onto the inner wall of the test-tube. Using a glass syringe, previously cooled to –10 °C, inject about 300 µl (corresponding to about 0.25 g) of liquid ethylene oxide R into 50 ml of macrogol 200 TS. Determine the absorbed quantity of ethylene oxide by weighing before and after absorption. Dilute to 100 ml with macrogol 200 TS. Mix well before use.
Assay. To 10 ml of a 500 g/l suspension of magnesium chloride R in dehydrated ethanol R add 20 ml of hydrochloric acid/ethanol (0.1 mol/l) VS, stopper the flask, shake to obtain a saturated solution, and allow to stand overnight to equilibrate. Weigh 5 g of the prepared ethylene oxide stock solution R (containing about 2.5 g/l) into the flask and allow to stand for 30 minutes. Titrate with potassium hydroxide/ethanol (0.1 mol/l) VS, determining the end-point potentiometrically. Carry out a blank titration, replacing the substance to be

examined with the same quantity of macrogol 200 TS. Calculate the content of ethylene oxide in mg/g.

Storage. Keep in a tightly closed container in a refrigerator at 4 °C.

Ethylenediamine R. $C_2H_8N_2$.

Description. A colourless to pale yellow, clear liquid; odour, ammonia-like.

Miscibility. Miscible with water and ethanol (~750 g/l) TS; slightly miscible with ether R.

Boiling temperature. About 116 °C.

Mass density. ρ_{20} = about 0.898 kg/l.

Storage. Store in a tightly closed container, protected from air and acidic vapours.

Ethylmethylketone R. C_4H_8O.

Description. A clear, colourless, mobile liquid; odour, characteristic.

Miscibility. Miscible with water, ethanol (~750 g/l) TS, and ether R.

Boiling range. 79–80 °C.

Mass density. ρ_{20} = about 0.805 kg/l.

1-Ethylquinaldinium iodide R. 1-Ethyl-2-methylquinolinium iodide; $C_{12}H_{14}IN$.

A commercially available reagent of suitable grade.

Description. A yellow-green solid.

Solubility. Sparingly soluble in water.

1-Ethylquinaldinium iodide (15 g/l) TS. A solution containing about 15 g of 1-ethylquinaldinium iodide R per litre.

Etoposide RS. International Chemical Reference Substance.

Ferric ammonium sulfate (0.1 mol/l) VS. Ferric ammonium sulfate R, dissolved in a mixture of sulfuric acid (~1760 g/l) TS and water to contain 48.22 g of $FeNH_4(SO_4)_2,12H_2O$ in 1000 ml.

Procedure. Dissolve 50 g of ferric ammonium sulfate R in a mixture of 300 ml of water and 6 ml of sulfuric acid (~1760 g/l) TS. Dilute with sufficient water to produce 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Transfer 25 ml to a glass-stoppered flask and add 3 ml of hydrochloric acid (~420 g/l) TS and 2 g of potassium iodide R. Allow the solution to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Perform a blank determination and make any necessary corrections.

Storage. Store this solution in a tightly closed container, protected from light.

Ferric ammonium sulfate (45 g/l) TS. A solution of ferric ammonium sulfate R containing about 45 g/l of $FeNH_4(SO_4)_2$.

Ferric ammonium sulfate R. $FeNH_4(SO_4)_2,12H_2O$ (SRIP, 1963, p. 88).

Ferric ammonium sulfate TS1

Procedure. Dissolve 0.2 g of ferric ammonium sulfate R in 50 ml of water, add 6 ml of nitric acid (~1000 g/l) TS and sufficient water to produce 100 ml.

Ferric ammonium sulfate TS2

Procedure. Dissolve 8.3 g of ferric ammonium sulfate R in sufficient sulfuric acid (0.25 mol/l) VS to produce 1000 ml.

Ferric chloride (25 g/l) TS. A solution of ferric chloride R containing about 27 g/l of $FeCl_3$.

Ferric chloride (50 g/l) TS. A solution of ferric chloride R containing about 50 g of $FeCl_3$ per litre.

Ferric chloride (65 g/l) TS. A solution of ferric chloride R containing about 65 g of $FeCl_3$ per litre.

Ferric chloride R. $FeCl_3,6H_2O$ (SRIP, 1963, p. 88).

Ferric chloride/ferricyanide/arsenite TS

Procedure. Prepare 3 separate solutions:

(1) Dissolve 2.7 g of ferric chloride R in 100 ml of hydrochloric acid (~70 g/l) TS.
(2) Dissolve 3.5 g of potassium ferricyanide R in 100 ml of water. This solution should be freshly prepared.
(3) Dissolve 3.8 g of arsenic trioxide R in 25 ml of hot sodium hydroxide (~80 g/l) TS. Allow to cool, add 50 ml of sulfuric acid (~100 g/l) TS, and dilute to 100 ml with water.

Immediately before use mix 5 volumes of solution (1), 5 volumes of solution (2), and 1 volume of solution (3).

Ferric chloride/potassium ferricyanide TS

Procedure. Dissolve 2 g of ferric chloride R and 0.10 g of potassium ferricyanide R in sufficient water to produce 20 ml.

Note. Ferric chloride/potassium ferricyanide TS must be freshly prepared.

Ferricyanide standard (50 µg/ml) TS

Procedure. Prepare a solution of potassium ferricyanide R in water to contain 7.8 g of $K_3Fe(CN)_6$ per 100 ml. Dilute 1.0 ml of this solution with sufficient water to produce 1000 ml.

Note. Ferricyanide standard (50 µg/ml) TS must be freshly prepared.

Ferrocyanide standard (100 µg/ml) TS

Procedure. Prepare a solution of potassium ferrocyanide R in water to contain 2.0 g of $K_4Fe(CN)_6,3H_2O$ per 100 ml. Dilute 1.0 ml of this solution with sufficient water to produce 100.0 ml.

Note. Ferrocyanide standard (100 µg/ml) TS must be freshly prepared.

Ferroin TS

Procedure. Dissolve 0.15 g of *o*-phenanthroline R in 10 ml of a solution of ferrous sulfate R, prepared by dissolving 0.70 g of clear crystals of ferrous sulfate R in 100 ml of water.

Note: The ferrous sulfate solution must be prepared immediately before dissolving the *o*-phenanthroline.

Storage. Ferroin TS should be stored in well-closed containers.

Ferrous ammonium sulfate (0.1 mol/l) VS

Procedure. Dissolve 40 g of ferrous ammonium sulfate R in 100 ml of sulfuric acid (~190 g/l) TS, and dilute to 1000 ml with carbon-dioxide-free water R.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: To 25 ml add 10 ml of sulfuric acid (~100 g/l) TS and 1 ml of phosphoric acid (~440 g/l) TS and titrate with potassium permanganate (0.02 mol/l) VS. Each ml of potassium permanganate (0.02 mol/l) VS is equivalent to 39.21 mg of $(NH_4)_2Fe(SO_4)_2$.

Ferrous ammonium sulfate (1 g/l) TS. A solution of ferrous ammonium sulfate R containing about 1 g/l of $Fe(NH_4)_2(SO_4)_2$.

Ferrous ammonium sulfate R. $Fe(NH_4)_2(SO_4)_2,6H_2O$ (SRIP, 1963, p. 89).

Ferrous sulfate (0.1 mol/l) VS

Procedure. Dissolve 2.8 g of ferrous sulfate R in 90 ml of freshly boiled and cooled water, and add a sufficient quantity of sulfuric acid (~1760 g/l) TS to produce 100 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: To 40.0 ml of the ferrous sulfate solution add 5 ml of phosphoric acid (~1,440 g/l) TS and titrate immediately with potassium permanganate (0.02 mol/l) VS.

Note: Standardize immediately before use.

Ferrous sulfate (15 g/l) TS. A solution of ferrous sulfate R in freshly boiled and cooled water containing about 15 g/l of $FeSO_4$ (approximately 0.1 mol/l).

Note: Ferrous sulfate (15 g/l) TS must be freshly prepared.

Ferrous sulfate (7 g/l) TS. A solution of ferrous sulfate R in freshly boiled and cooled water containing about 7 g of $FeSO_4$ per litre.

Note: Ferrous sulfate (7 g/l) TS must be freshly prepared.

Ferrous sulfate R. $FeSO_4,7H_2O$ (SRIP, 1963, p. 90).

Ferrous sulfate/hydrochloric acid TS
Procedure. Dissolve 0.45 g of ferrous sulfate R in 50 ml of hydrochloric acid (0.1 mol/l) VS and dilute with sufficient carbon-dioxide-free water R to produce 100 ml.
Note: Ferrous sulfate/hydrochloric acid TS should be prepared immediately before use.

Firebrick, pink, R. A suitable grade for use in gas chromatography with an average particle size of about 180–250 µm.

Flucloxacillin sodium RS. International Chemical Reference Substance.

Flucytosine RS. International Chemical Reference Substance.

Fludrocortisone acetate RS. International Chemical Reference Substance.

Fluoroquinolonic acid RS. International Chemical Reference Substance.

Fluorouracil RS. International Chemical Reference Substance.

Fluphenazine decanoate RS. International Chemical Reference Substance.

Fluphenazine enantate RS. International Chemical Reference Substance.

Fluphenazine hydrochloride RS. International Chemical Reference Substance.

Folic acid RS. International Chemical Reference Substance.

Formaldehyde TS [formaldehyde R]. (SRIP, 1963, p. 91).

Formaldehyde/sulfuric acid TS
Procedure. To 10 ml of sulfuric acid (~1760 g/l) TS add 0.2 ml of formaldehyde TS.
Shelf-life. Use within 1 month after preparation.

Formamide R. CH_3NO (SRIP, 1963, p. 92).

Formic acid (~1080 g/l) TS
[formic acid R]. CH_2O_2 (SRIP, 1963, p. 92) $d \sim 1.2$.

Formic acid, anhydrous, R. CH_2O_2, $d \sim 1.22$. Contains not less than 98.0% of CH_2O_2.
Description. A colourless liquid; odour, pungent.

Miscibility. Miscible with water and ethanol (~750 g/l) TS.

Chlorides. Dilute 1 ml to 15 ml with water and proceed as described under 2.2.1 Limit test for chlorides. Anhydrous formic acid R contains not more than 0.50 mg/g.

Sulfates. Dilute 0.5 ml to 15 ml with water and proceed as described under 2.2.2 Limit test for sulfates. Anhydrous formic acid R contains not more than 1.5 mg/g.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; leaves not more than 0.5 mg/g of residue.

Assay. To a tared flask containing about 10 ml of water, quickly add about 1 ml of the test liquid, and weigh. Dilute with 50 ml of water, and titrate with carbonate-free sodium hydroxide (1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 46.03 mg of CH_2O_2.

3-Formylrifamycin SV RS. International Chemical Reference Substance.

Framycetin sulfate RS. International Chemical Reference Substance.

Fuchsin TS

Procedure. Pour carefully 40 ml of sulfuric acid (~1760 g/l) TS into 60 ml of water. Allow to cool and add 100 ml of a 1 g/l solution of basic fuchsin R. Dilute with water to 200 ml and allow to stand. An orange-yellow colour develops. Immediately before use dilute the solution with an equal volume of glacial acetic acid R.

Fuchsin, basic, R [magenta, basic R]. A mixture of rosaniline hydrochloride, $(H_2NC_6H_4)_2C:C_6H_3(CH_3):NH_2^+Cl^-$, and pararosaniline hydrochloride, $(H_2NC_6H_4)_2C:C_6H_4:NH_2^+Cl^-$.

Description. Crystals or crystalline fragments, with a glossy, greenish-bronze lustre.

Solubility. Soluble in water, ethanol (~750 g/l) TS, and amyl alcohol R.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 0.10 g/g.

Sulfated ash. Ignite 1 g with 0.5 ml of sulfuric acid (~1760 g/l) TS: not more than 3.0 mg/g.

Fuchsin, decolorized, TS

Procedure. Dissolve 1 g of basic fuchsin R in 600 ml of water and cool in an ice-bath; add 20 g of sodium sulfite R dissolved in 100 ml of water; cool in an ice-bath and add slowly, with constant stirring, 10 ml of hydrochloric acid (~250 g/l) TS; dilute with water to 1000 ml. If the resulting solution is turbid, it should be filtered and, if brown in colour, it should be shaken with sufficient charcoal R (0.2–0.3 g) to render it colourless, and then filtered immediately. Occasionally, it is necessary to add 2–3 ml of hydrochloric acid (~250 g/l) TS, followed by shaking, to remove a little residual pink colour.

The solution resulting from any of the foregoing modifications should be allowed to stand overnight before use. Decolorized fuchsin TS should be protected from light.

Fuchsin/sulfurous acid TS

Procedure. Dissolve 0.10 g of fuchsin, basic, R in 50 ml of water with gentle heating. To the cooled solution add 20 ml of sodium metabisulfite (50 g/l) TS and 1 ml of hydrochloric acid (~420 g/l) TS. Dilute to 100 ml with water, mix, and allow to stand in the dark for 2 hours. Fuchsin/sulfurous acid TS should be colourless and should not be used for a period longer than 24 hours.

Furosemide RS. International Chemical Reference Substance.

Gallamine triethiodide RS. International Chemical Reference Substance.

Gelatin R. Gelatin of suitable purity.

Gelatin TS. A solution of gelatin R dissolved in phosphate buffer, pH 7.0, TS containing about 10 g/l.

Gentamicin sulfate RS. International Chemical Reference Substance.

Glibenclamide RS. International Chemical Reference Substance.

Glucose hydrate R. Monohydrate of α-D-glucopyranose, $C_6H_{12}O_6,H_2O$. Contains not less than 99.0% and not more than 101.5% of $C_6H_{12}O_6$, calculated with reference to the dried substance.

Description. Colourless crystals or a white crystalline or granular powder; odourless.

Solubility. Soluble in about 1 part of water and in about 60 parts of ethanol (~750 g/l) TS; more soluble in boiling water and in boiling ethanol (~750 g/l) TS.

Acidity. Dissolve 5 g in 50 ml of carbon-dioxide-free water R. It requires for neutralization not more than 0.5 ml of carbonate-free sodium hydroxide (0.02 mol/l) VS, phenolphthalein/ethanol TS being used as indicator.

Specific optical rotation. Dissolve 100 mg, previously dried to constant weight, in 1 ml of water, and add a few drops of ammonia (~100 g/l) TS; $[\alpha]_D^{20\,°C} = +52$ to $+53\,°$.

Soluble starch or sulfites. Dissolve 1 g in 10 ml of water and add 1 drop of iodine TS; the liquid is coloured yellow.

Loss on drying. Dry to constant weight at 105 °C; it loses not less than 80 mg/g and not more than 100 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Assay. Dissolve about 0.1 g, accurately weighed, in 50 ml of water, add 30 ml of iodine (0.1 mol/l) VS, 10 ml of sodium carbonate (50 g/l) TS, and allow to

stand for 20 minutes. Add 15 ml of hydrochloric acid (~70 g/l) TS and titrate the excess of iodine with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Perform a blank determination and make any necessary corrections. Each ml of iodine (0.1 mol/l) VS is equivalent to 9.008 mg of $C_6H_{12}O_6$.

Glucose, anhydrous, R. $C_6H_{12}O_6$. Use anhydrous glucose as described in the monograph for "Glucose".

Glycerol R. Propane-1,2,3-triol with small amounts of water, $C_3H_8O_3$. Contains not less than 970 g/kg of $C_3H_8O_3$.

Description. A clear, almost colourless, syrupy and hygroscopic liquid; odourless.

Miscibility. Miscible with water and ethanol (~750 g/l) TS; practically immiscible with ether R.

Mass density(ρ_{20}). Not less than 1.256 kg/l.

Refractive index(n_D^{20}). Not less than 1.469.

Acrolein and other reducing substances. Mix 1 ml with 1 ml of ammonia (~100 g/l) TS and heat in a water-bath at 60 °C for 5 minutes; the liquid is not coloured yellow. Remove from the water-bath and add 3 drops of silver nitrate (40 g/l) TS; the liquid does not become coloured within 5 minutes.

Sulfated ash. Not more than 0.5 mg/ml.

Glycine R. Aminoacetic acid; $C_2H_5NO_2$.

Description. A white, crystalline powder.

Solubility. Very soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Assay. Determine the nitrogen by the Kjeldahl method in the test substance previously dried at 105 °C for 2 hours; between 18.4 and 18.8% of N is found, corresponding to not less than 98.6% and not more than the equivalent of 100.8% of $C_2H_5NO_2$.

Insoluble matter. 10 g shows not more than 1.0 mg of insoluble matter (0.1 mg/g).

Sulfated ash. Not more than 0.5 mg/g.

Chlorides. Not more than 0.1 mg of Cl/g.

Sulfates. Not more than 0.05 mg of SO_4/g.

Heavy metals. Not more than 0.02 mg/g.

Iron. Not more than 0.01 mg of Fe/g, 3 ml of hydrochloric acid (~420 g/l) TS being used to facilitate solution.

Glyoxal bis(2-hydroxyanil) R. 2,2′-(Ethanediylidenedinitrilo)diphenol, $C_{14}H_{12}N_2O_2$.

Description. White crystals.

Solubility. Soluble in hot ethanol (~750 g/l) TS.

Melting temperature. 203–205 °C.

Glyoxal bis(2-hydroxyanil) TS. A solution of glyoxal bis(2-hydroxyanil) R dissolved in ethanol (~750 g/l) TS containing about 10 g/l of $C_{14}H_{12}N_2O_2$.

Green stock standard TS
Procedure. To 3.5 ml of cobalt colour TS, add 20.1 ml of copper colour TS, 10.4 ml of dichromate colour TS, and 4.0 ml of iron colour TS; dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Griseofulvin RS. International Chemical Reference Substance.

Haloperidol RS. International Chemical Reference Substance.

Helium R. He. Contains not less than 999.95 ml/l of He.

Heparin RS. World Health Organization International Reference Material. Heparin, porcine, mucosal. 5th International Standard 1998. (Ampoules containing 2031 IU (distributed by the National Institute for Biological Standards and Control (NIBSC), PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QH, England.))

Heparinized saline TS. A sterile solution of saline TS containing 50 International Units of heparin in 1 ml.

Heptane R. C_7H_{16} (SRIP, 1963, p. 94).

Hexamethyldisilazane R. $C_6H_{19}NSi_2$.
Description. A clear, colourless liquid, having a characteristic odour.
Mass density. ρ_{20} = about 0.77 kg/l.

Hexane R. *n*-Hexane, C_6H_{14}.
Description. A colourless, mobile, highly inflammable liquid.
Boiling range. Distils completely over a range of 1 °C between 67.5 and 69.5 °C.
Mass density. ρ_{20} = 0.658–0.659 kg/l
Refractive index. n_D^{20} = 1.374 – 1.375.

Hexylamine R. Hexaneamine; $C_6H_{15}N$.
A commercially available reagent of suitable grade.
Description. A colourless liquid.
Boiling point. 127–131 °C.
Refractive index. n_D^{20} = about 1.418.
Mass density. ρ_{20} = about 0.766 kg/l.

Histamine dihydrochloride R. $C_5H_9N_3,2HCl$. Contains not less than 98.0%, and not more than 101.0% of $C_5H_9N_3,2HCl$, calculated with reference to the dried substance.
Description. Colourless crystals or a white crystalline powder; odourless.
Solubility. Freely soluble in water and in methanol R; soluble in ethanol (~750 g/l) TS.
Melting range. 244–246 °C.

Loss on drying. Not more than 5.0 mg/g.

Assay. Dissolve about 0.15 g, accurately weighed, in 10 ml of water. Add 5 ml of chloroform R and 25 ml of ethanol (~750 g/l) TS. Titrate with carbonate-free sodium hydroxide (0.2 mol/l) VS, using 0.5 ml of thymolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (0.2 mol/l) VS is equivalent to 9.21 mg of $C_5H_9N_3,2HCl$.

Histamine phosphate R. $C_5H_9N_3,2H_3PO_4$. Contains not less than 98.0% and not more than 101.0% of $C_5H_9N_3,2H_3PO_4$, calculated with reference to the anhydrous substance.

Description. Colourless, long, prismatic crystals; odourless. Stable in air.

Solubility. Soluble in about 5 parts of water; slightly soluble in ethanol (~750 g/l) TS.

Melting temperature. About 132 °C.

Water. Determined by the Karl Fischer method, using about 1.0 g, the water content is 50–60 mg/g.

Assay. Dissolve about 0.15 g, accurately weighed, in 10 ml of water. Add 5 ml of chloroform R and 25 ml of ethanol (~750 g/l) TS. Titrate with carbonate-free sodium hydroxide (0.2 mol/l) VS, using 0.5 ml of thymolphthalein/ethanol TS as indicator. Each ml of carbonate-free sodium hydroxide (0.2 mol/l) VS is equivalent to 15.36 mg of $C_5H_9N_3,2H_3PO_4$.

Histamine TS. A solution containing 1.0 mg/l of histamine base.

Procedure. Prepare histamine TS by diluting strong histamine TS with a sufficient quantity of saline TS.

Note: Histamine TS must be freshly prepared.

Histamine, strong, TS. A solution containing 1.00 g/l of histamine base.

Procedure. Dissolve 138.1 mg, accurately weighed, of histamine phosphate R or 82.8 mg, accurately weighed, of histamine dihydrochloride R in sufficient water to produce 50.0 ml.

Storage. Strong histamine TS should be stored at a temperature not exceeding 4–10 °C, in dark glass bottles with ground-glass stoppers, protected from light.

Shelf-life. Do not use longer than 30 days.

Holmium oxide R. Ho_2O_3. Contains not less than 99.9% of Ho_2O_3, the impurities consisting of Er_2O_3 and Dy_2O_3.

Description. A tan-coloured powder.

Solubility. Insoluble in water.

Holmium perchlorate TS

Procedure. Dissolve 40 g of holmium oxide R in sufficient perchloric acid (~140 g/l) TS to produce 1000 ml.

Hydrazine hydrate R. N_2H_4,H_2O. Contains not less than 98.0% of N_2H_4,H_2O.

Description. A clear, colourless liquid.

Miscibility. Miscible with water.
Residue on evaporation. Evaporate to dryness on a water-bath; it leaves a residue of not more than 5.0 mg/g.
Assay. Dilute 1 g to 200 ml with water. Neutralize 20 ml of this solution with hydrochloric acid (~420 g/l) TS and add 10 ml in excess. Add 5 ml of potassium cyanide (100 g/l) TS, titrate with potassium iodate (0.05 mol/l) VS until the brown colour which first forms becomes pale, add starch TS, and continue the titration until the blue colour disappears. Each ml of potassium iodate (0.05 mol/l) VS is equivalent to 2.503 mg of N_2H_4,H_2O.

Hydrazine sulfate R. $(NH_4)_2,H_2SO_4$.
Description. Colourless crystals or a white, crystalline powder.
Solubility. Soluble in about 40 parts of water; practically insoluble in ethanol (~750 g/l) TS.
Arsenic. Use a solution of 10 g in 35 ml of boiling water and proceed as described under 2.2.5 Limit test for arsenic; not more than 1 µg/g.
Sulfated ash. Not more than 1.0 mg/g.

Hydriodic acid (~970 g/l) TS [hydriodic acid R]. HI (SRIP, 1963, p. 95).

Hydrochloric acid (~330 g/l) TS. A solution of hydrochloric acid (~420 g/l) TS in water, containing approximately 330 g of HCl per litre; $d \sim 1.15$ (about 9 mol/l).

Hydrochloric acid (~420 g/l) TS [hydrochloric acid, saturated, R]. (SRIP, 1963, p. 96); $d \sim 1.18$.

Hydrochloric acid (~250 g/l) AsTS. Hydrochloric acid (~250 g/l) TS that complies with the following tests A and B:

A. Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of ammonium thiocyanate (75 g/l) TS and stir immediately; no colour is produced.
B. To 50 ml add 0.2 ml of bromine AsTS, evaporate in a water-bath until reduced to 16 ml, adding more bromine AsTS if necessary to ensure that an excess, as indicated by the colour, is present throughout the evaporation. Add 50 ml of water and 5 drops of stannous chloride AsTS and apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 0.2-ml standard stain, showing that the amount of arsenic does not exceed 0.05 µg/ml.

Hydrochloric acid (~250 g/l) FeTS. Hydrochloric acid (~250 g/l) TS that complies with the following additional test: Evaporate 5 ml nearly to dryness on a water-bath, add 40 ml of water, 2 ml of citric acid (180 g/l) FeTS, and 2 drops of mercaptoacetic acid R; mix, make alkaline with ammonia (~100 g/l) FeTS, and dilute to 50 ml with water; no pink colour is produced.

Hydrochloric acid (~250 g/l), stannated, AsTS
Procedure. Dilute 1 ml of stannous chloride AsTS with sufficient hydrochloric acid (~250 g/l) AsTS to produce 100 ml.

Hydrochloric acid (~70 g/l) TS
Procedure. Dilute 260 ml of hydrochloric acid (~250 g/l) TS with sufficient water to produce 1000 ml (approximately 2 mol/l); $d \sim 1.035$.

Hydrochloric acid (0.0001 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 3.647 mg of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.001 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 36.47 mg of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.005 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 0.1824 g of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.01 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 0.3647 g of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.015 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 0.5470 g of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.02 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 0.7293 g of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.05 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 1.824 g of HCl in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.1 mol/l) BET. Prepare from hydrochloric acid (~420 g/l) TS and water BET. It is suitable if, after adjustment to pH 6.5–7.5, it gives

a negative result under the conditions prescribed in the 3.4 Test for bacterial endotoxins.

Hydrochloric acid (0.1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 3.647 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.2 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 7.293 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (0.5 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 18.23 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 36.47 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: Dissolve about 1.5 g, accurately weighed, of anhydrous sodium carbonate R, previously dried at 270 °C for 1 hour, in 50 ml of water and titrate with the hydrochloric acid solution, using methyl orange/ethanol TS as indicator. Each 52.99 mg of anhydrous sodium carbonate is equivalent to 1 ml of hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (2 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 72.93 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (5 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with water to contain 182.35 g of HCl in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid (~250 g/l) TS. A solution of hydrochloric acid (~420 g/l) TS in water, containing approximately 250 g/l of HCl; $d \sim 1.12$.

Hydrochloric acid CITS. One millilitre contains 50 µg of Cl.

Procedure. Dilute 14.3 ml of hydrochloric acid (0.1 mol/l) VS with sufficient water to produce 1000 ml.

Hydrochloric acid, brominated, AsTS
Procedure. To 100 ml of hydrochloric acid (~250 g/l) AsTS add 1 ml of bromine AsTS.

Hydrochloric acid/ethanol (1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with dehydrated ethanol R to contain 36.47 g of HCl in 1000 ml of dehydrated ethanol R.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid/ethanol (0.1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with dehydrated ethanol R to contain 3.647 g of HCl in 1000 ml of dehydrated ethanol R.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochloric acid/methanol (0.01 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with methanol R to contain 0.3647 g of HCl in 1000 ml of methanol R.
Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS.

Hydrochlorothiazide RS. International Chemical Reference Substance.

Hydrocortisone acetate RS. International Chemical Reference Substance.

Hydrocortisone R. $C_{21}H_{30}O_5$. Use Hydrocortisone as described in the monograph for "Hydrocortisone".

Hydrocortisone RS. International Chemical Reference Substance.

Hydrocortisone sodium succinate RS. International Chemical Reference Substance.

Hydrogen peroxide (~330 g/l) TS [hydrogen peroxide (30 per cent.) R]. (SRIP, 1963, p. 97).

Hydrogen peroxide (~60 g/l) TS. A solution in water containing about 60 g of H_2O_2 per litre.

Hydrogen sulfide R. H_2S (SRIP, 1963, p. 98).

Hydrogen sulfide TS. A saturated solution of hydrogen sulfide R in cold water.
Note: Hydrogen sulfide TS must be freshly prepared.

Hydroquinone R. $C_6H_4(OH)_2$.
Description. Colourless or almost colourless crystals or a crystalline powder.
Solubility. Soluble in water, ethanol (~750 g/l) TS, and ether R.
Melting temperature. About 173 °C.
Note. Hydroquinone R darkens on exposure to air and light.

1-Hydroxy-9-anthrone RS. International Chemical Reference Substance.

(–)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine RS. International Chemical Reference Substance.

(–)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine RS. International Chemical Reference Substance.

Hydroxyethylcellulose R. Contains not less than 20% of $C_2H_5O_2$, calculated with reference to the dried substance.
Description. A white or yellowish, flaky, heterogeneous mass; odourless.
Solubility. Practically insoluble in ethanol (~750 g/l) TS; after soaking for several hours in water, freely soluble in water.
Colour of solution. Transfer 2 g to a 200-ml glass-stoppered, conical flask, add 200 ml of carbon-dioxide-free water R, shake, and allow to stand for 30 minutes. Repeat this operation until the substance has dissolved and filter through sintered glass. Observe 5 ml of the filtrate; it is colourless (keep the filtrate for the acidity or alkalinity test).
Loss on drying. To 1.0 g add 25 ml of water, stir, and allow to stand. Repeat this operation until dissolved. Evaporate on a water-bath and dry to constant weight at 110 °C; it loses not more than 0.10 g/g. (Keep the dried substance for the assay.)
Acidity or alkalinity. To 10 ml of the filtrate obtained from the test for colour of solution, add 2 drops of bromothymol blue/ethanol TS; a yellow colour is produced. Add 0.5 ml of potassium hydroxide (0.01 mol/l) VS; a green or blue solution is produced.
Assay. Place into a boiling flask as described under 2.9 Determination of methoxyl, 0.5 ml of acetic anhydride R, 0.05–0.10 g of phenol R, 0.20 g of red phosphorus R, and 5.0 ml of hydriodic acid (~970 g/l) TS; connect the flask to the condenser, pass a slow, uniform stream of carbon dioxide R through the solution, and heat for 60 minutes. Cool for 10 minutes and add 0.035 g, accurately weighed, of the dried substance obtained in the test for loss on drying. Proceed with this mixture as described under 2.9 Determination of methoxyl. For the calculation, take an average of 3 determinations. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 1.018 mg of $C_2H_5O_2$.

Hydroxyethylcellulose TS
Procedure. Place 50 ml of water in a 100 ml beaker and add 2.0 g of hydroxyethylcellulose R. After 15 hours, stir the solution for 1 minute and cen-

trifuge for 15 minutes. Using a pipette, separate 20 ml of the supernatant liquid.

Note: Hydroxyethylcellulose TS must be freshly prepared.

Hydroxylamine hydrochloride (200 g/l) TS. A solution of hydroxylamine hydrochloride R containing about 200 g of NH_2OH,HCl per litre.

Hydroxylamine hydrochloride (70 g/l) TS

Procedure. Dissolve 69.5 g of hydroxylamine hydrochloride R in sufficient water to produce 1000 ml (1 mol/l).

Hydroxylamine hydrochloride R. NH_2OH,HCl (SRIP, 1963, p. 99).

Hydroxylamine hydrochloride TS

Procedure. Dissolve 1 g of hydroxylamine hydrochloride R in 50 ml of water and add 50 ml of ethanol (~750 g/l) TS and 1 ml of bromophenol blue/ethanol TS; then add sodium hydroxide (0.1 mol/l) VS until the solution becomes green.

Hydroxylamine hydrochloride TS2

Procedure. Dissolve 3.5 g of hydroxylamine hydrochloride R in 95 ml of ethanol (~535 g/l) TS, add 0.5 ml of bromophenol blue (1 g/l) TS and sufficient potassium hydroxide/ethanol (0.5 mol/l) TS until a greenish tint is developed.

Dilute the solution to 100 ml with ethanol (~535 g/l) TS.

8-Hydroxyquinoline R. 8-Ouinolinol; C_9H_7NO.

Description. A white to yellowish white, crystalline powder.

Solubility. Practically insoluble in water and ether R; freely soluble in ethanol (~750 g/l) TS, and acetone R.

Melting point. About 74 °C.

8-Hydroxyquinoline/chloroform TS

Procedure. Dissolve 1 g of 8-hydroxyquinoline R in sufficient chloroform R to produce 100 ml.

Hypophosphorous acid R. Phosphinic acid; H_3PO_2 (SRIP, 1963, p. 100).

Hypophosphorous acid, dilute, TS. A solution of hypophosphorous acid R containing about 100 g of H_3PO_2 per 1000 ml.

Hypoxanthine R. 1,7-dihydro-6*H*-purin-6-one; $C_5H_4N_4O$.

A commercially available reagent of suitable grade.

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water, sparingly soluble in boiling water, soluble in dilute acids and in dilute alkali hydroxide solutions.

Melting point. Decomposes without melting at about 150 °C.

Thin-Layer Chromatography. Examine as prescribed in the monograph on Mercaptopurine; the chromatogram shows only one principal spot.

Ibuprofen RS. International Chemical Reference Substance.

Idoxuridine RS. International Chemical Reference Substance.

Imidazole R. Glyoxaline, $C_3H_4N_2$. Contains not less than 99.0% of $C_3H_4N_2$.
Description. A white, crystalline powder.
Solubility. Soluble in water and ethanol (~750 g/l) TS.
Melting range. 89–93 °C.
Sulfated ash. Not more than 0.5 mg/g.
Assay. Dissolve 0.3 g in 50 ml of water and titrate with sulfuric acid (0.05 mol/l) VS, using bromocresol green/ethanol TS as indicator. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 6.808 mg of $C_3H_4N_2$.

Imidazole, recrystallized, R
Procedure. Dissolve 25 g of imidazole R in 100 ml of hot toluene R and cool in an ice-bath, while stirring. Filter off the crystals with suction using filter-paper Whatman No. 54 or No. 541. Repeat the crystallization and filtration, sucking as dry as possible. Slurry wash the resulting crystals with about 50 ml of ether R and filter. Repeat this process and then wash the crystals on the filter with ether R and suck as dry as possible. Transfer to a shallow dish and dry at room temperature under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over silica gel, desiccant, R.
Storage. Store in a tightly closed container.

Imidazole/mercuric chloride TS
Procedure. Dissolve 8.25 g of recrystallized imidazole R in 60 ml of water and add 10 ml of hydrochloric acid (5 mol/l) VS. Under continuous stirring add, drop by drop, 10 ml of mercuric chloride (2.7 g/l) TS. If a cloudy solution results, discard and prepare a further solution by adding the mercuric chloride solution more slowly. Adjust the pH to 6.80 ± 0.05 with hydrochloric acid (5 mol/l) VS (about 4 ml is required) and add sufficient water to produce 100 ml.

Iminodibenzyl R. 10,11-Dihydro-5*H*-dibenz[*b,f*]azepine; $C_{14}H_{13}N$.
Description. A pale yellow, crystalline powder.
Melting temperature. About 106 °C.

Imipramine hydrochloride RS. International Chemical Reference Substance.

Indinavir RS. International Chemical Reference Substance.

Indometacin RS. International Chemical Reference Substance.

Iodide standard (20 µg I/ml) TS

Procedure. Dissolve 26.0 mg of potassium iodide R in sufficient water to produce 100 ml. Dilute 10 ml of this solution to 100 ml with water.

Iodine (0.0001 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 25.38 mg of I_2 and 36 mg of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine (0.0005 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 0.127 g of I_2 and 0.18 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Iodine (0.1 mol/l) VS".

Iodine (0.005 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 1.269 g of I_2 and 1.80 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine (0.01 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 2.538 g of I_2 and 3.6 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine (0.02 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 5.076 g of I_2 and 7.2 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine (0.05 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 12.69 g of I_2 and 18.0 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under iodine (0.1 mol/l) VS.

Iodine (0.1 mol/l) VS. Iodine R and potassium iodide R, dissolved in water to contain 25.38 g of I_2 and 36.0 g of KI in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution by titrating 25.0 ml with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator.

Iodine bromide R. IBr.

Description. Blue-black or brownish-black crystals.

Solubility. Freely soluble in water, ethanol (~750 g/l) TS, ether R, and glacial acetic acid R.

Melting temperature. About 40 °C.

Storage. Store in a cool place, in a tightly closed container, protected from light.

Iodine bromide TS

Procedure. Dissolve 20 g of iodine bromide R in sufficient glacial acetic acid R to produce 1000 ml.

Storage. Store in a tightly closed container, protected from light.

Iodine pentoxide R. Iodic anhydride; I_2O_5.

A commercially available reagent of suitable grade.

Iodine R. I_2 (SRIP, 1963, p. 101).

Iodine TS

Procedure. Dissolve 2.6 g of iodine R and 3 g of potassium iodide R in sufficient water to produce 100 ml (approximately 0.1 mol/l).

Iodine/chloroform TS

Procedure. Dissolve 5.0 g of iodine R in sufficient chloroform R to produce 100 ml.

Iodine/ethanol TS

Procedure. Dissolve 10 g of iodine R in sufficient ethanol (~750 g/l) TS to produce 1000 ml.

Iohexol RS. International Chemical Reference Substance.

Iopanoic acid RS. International Chemical Reference Substance.

Iotroxic acid RS. International Chemical Reference Substance.

Iron colour TS. A solution containing 45.0 mg/ml of $FeCl_3,6H_2O$.

Procedure. Prepare a solution containing 4.500 g of $FeCl_3,6H_2O$ in 100 ml, diluting the strong iron colour TS with sulfuric acid (~10 g/l) TS, as necessary.

Iron colour, strong, TS

Procedure. Dissolve 6.6 g of ferric chloride R in 120 ml of sulfuric acid (~10 g/l) TS, filter the solution, if necessary, and determine the concentration of $FeCl_3,6H_2O$.

Assay. Dilute 5.0 ml with sufficient water to produce 25.0 ml. Transfer 10.0 ml of this solution to a flask, and add 60 ml of water. Adjust the pH to 2–3 with hydrochloric acid (1 mol/l) VS and ammonia (~100 g/l) TS, using congo red paper R. Heat the solution to approximately 45 °C, and titrate with disodium edetate (0.05 mol/l) VS, using 2 ml of sulfosalicylic acid (175 g/l) TS as indicator, until the solution changes from a lilac tint to straw-yellow. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 13.52 mg of $FeCl_3,6H_2O$.

Iron salicylate TS

Procedure. Dissolve 0.5 g of ferric ammonium sulfate R in 250 ml of water containing 10 ml of sulfuric acid (~100 g/l) TS and dilute with sufficient water to produce 500 ml. To 100 ml of this solution add 50 ml of sodium salicylate (11.5 g/l) TS, 20 ml of acetic acid (~60 g/l) TS and 80 ml of sodium acetate (150 g/l) TS, and dilute with water to 500 ml.

Storage. Store in a well-closed container, protected from light.

Note. Iron salicylate must be freshly prepared.

Iron standard FeTS

Procedure. Dissolve 0.173 g of ferric ammonium sulfate R in 100 ml of water, add 5 ml of hydrochloric acid (~70 g/l) TS and sufficient water to produce 1000 ml. Each ml of this solution contains 20 µg of iron.

Iron, reduced, R. Fe (SRIP, 1963, p. 102).

Isobutyl methyl ketone R. 4-Methyl-2-pentanone; $C_6H_{12}O$.

Description. A clear, colourless liquid; odour, characteristic.

Boiling point. About 115 °C.

Mass density. ρ_{20} = about 0.80 kg/l.

Isoniazid R. Isoniazid as described in the monograph for "Isoniazid".

Isoniazid RS. International Chemical Reference Substance.

Isoniazid TS

Procedure. Dissolve 0.1 g of isoniazid R in 150 ml of methanol R, add 0.12 ml of hydrochloric acid (~420 g/l) TS, and dilute with methanol R to 200 ml.

Isopropylamine R. C_3H_9N.

Description. A colourless, volatile liquid with an ammoniacal odour.

Boiling point. About 33 °C.

Mass density. ρ_{20} = about 0.69 kg/l.

Kanamycin monosulfate RS. International Chemical Reference Substance.

Kaolin, light, R. Kaolin as described in the monograph for "Kaolin".

Kaolin suspension TS

Procedure. Immediately before use mix equal volumes of cephalin TS and a suspension containing 4 g of kaolin R in 1000 ml of sodium chloride (9 g/l) TS.

Karl Fischer reagent TS. A freshly prepared solution contains up to 5.0 mg/ml of water. The solution should not be used if the water equivalent falls below 2.5 mg of water per ml of the reagent.

Procedure. Dissolve 63 g of iodine R in 100 ml of anhydrous pyridine R, cool in ice, and pass sulfur dioxide R into the solution until a gain in weight of 32 g has occurred, taking care to avoid absorption of atmospheric moisture. Add sufficient dehydrated methanol R to produce 500 ml and allow to stand for 24 hours. Karl Fischer reagent TS may also be prepared by mixing commercially available solutions of sulfur dioxide in pyridine and of iodine in methanol, which are stable when properly stored, for example, protected from light. The resulting solution should conform to the requirements stated below.

Method of standardization. Ascertain the exact content of water in the following manner: Add about 20 ml of dehydrated methanol R, to the titration vessel and titrate to the endpoint with Karl Fischer reagent TS without recording the volume required. Introduce in an appropriate form a suitable amount of water, accurately weighed, and titrate again to the endpoint with Karl Fischer reagent TS, recording the volume. Water might be introduced, for example, as a solution in dry methanol, or under the form of a hydrated compound. Calculate the water equivalent of the reagent in mg of water per ml. Karl Fischer reagent TS deteriorates continuously and should be standardized immediately before use, or daily, as required.

Note: Ethylene glycol monoethyl ether R may be used in the preparation of the reagent instead of dehydrated methanol R.

Ketamine hydrochloride RS. International Chemical Reference Substance.

Ketoconazole RS. International Chemical Reference Substance.

Kieselguhr R1. Kieselguhr G.

Description. A greyish-white powder, of an average particle size between 10 and 40 µm, containing per kg about 150 g of calcium sulfate, hemihydrate.

Kieselguhr R2. Kieselguhr GF254.

Description. A greyish-white powder, of an average particle size between 10 and 40 µm, containing per kg about 150 g of calcium sulfate, hemihydrate and an adequate amount (usually about 15 g/kg) of a fluorescent indicator having a maximum absorption at 254 nm.

Kieselguhr R3

Description. A greyish-white powder, of an average particle size between 170 and 200 µm.

Kieselguhr R4

Description. A greyish-white powder, of an average particle size between 70 and 150 µm.

Kieselguhr R5. Kieselguhr H.

Description. A fine, greyish-white powder; the grey colour becomes more pronounced on triturating the powder with water. The average particle size is between 10 and 40 µm.

Lactobionic acid R. 4-O-β-D-Galactopyranosyl-D-gluconic acid; $C_{12}H_{22}O_{12}$. A commercially available reagent of suitable grade.

Lanthanum nitrate (30 g/l) TS

Procedure. Dissolve 4.3 g of lanthanum nitrate R in 1 ml of nitric acid (~130 g/l) TS and sufficient water to produce 100 ml.

Lanthanum nitrate R. $La(NO_3)_3,6H_2O$. Contains not less than 97.0% of $La(NO_3)_3,6H_2O$.

Description. Colourless crystals; deliquescent.

Solubility. Freely soluble in water.

Assay. Dissolve about 0.75 g, accurately weighed, in 25 ml of water, add 3 ml of nitric acid (~130 g/l) TS, 3 g of methenamine R, and about 20 mg of xylenol orange indicator mixture R, and titrate with disodium edetate (0.05 mol/l) VS until the solution becomes pure yellow in colour. If fading of the colour of the indicator occurs towards the end of the titration, more methenamine R should be added. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 21.65 mg of $La(NO_3)_3,6H_2O$.

Lead acetate (80 g/l) TS. A solution of lead acetate R in freshly boiled water containing about 80 g/l of $C_4H_6O_4Pb$ (approximately 0.25 mol/l).

Lead acetate paper R

Procedure. Dip white filter-paper into a mixture of 10 volumes of lead acetate (80 g/l) TS and 1 volume of acetic acid (~60 g/l) TS. Allow to dry and cut the paper into strips measuring 15 mm × 40 mm.

Storage. Lead acetate paper R should be kept in a well-closed container.

Lead acetate R. $C_4H_6O_4Pb,3H_2O$ (SRIP, 1963, p. 105).

Lead nitrate (0.05 mol/l) VS. Lead nitrate R, dissolved in water to contain 16.56 g of $Pb(NO_3)_2$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/l solution by diluting 25.0 ml with 200 ml of water, add 10 ml of ammonia buffer TS and about 20 mg of Mordant Black 11 indicator mixture R, and titrate with disodium edetate (0.05 mol/l) VS.

Lead nitrate (0.1 mol/l) VS. Lead nitrate R, dissolved in water to contain 33.12 g of $Pb(NO_3)_2$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Lead nitrate (0.05 mol/l) VS".

Lead nitrate (100 g/l) TS. A solution of lead nitrate R containing 100 g of $Pb(NO_3)_2$ per litre.

Lead nitrate paper R

Procedure. Dip strips of suitable filter-paper in a solution of 10 g of lead nitrate R in 100 ml of water, and allow to dry.

Lead nitrate R. $Pb(NO_3)_2$ (SRIP, 1963, p. 107).

Lead subacetate TS. Contains not less than 16.7% *m/m* and not more than 17.4% *m/m* of Pb in a form corresponding approximately to the formula $C_8H_{14}O_{10}Pb_3$.

Procedure. Dissolve 40.0 g of lead acetate R in 90 ml of carbon-dioxide-free water R. Adjust the pH to 7.5 with sodium hydroxide (~400 g/l) TS. Centrifuge and use the clear supernatant solution.

Storage. Lead subacetate TS should be stored in a well-closed container.

Lead(IV) oxide R. PbO_2 (SRIP, 1963, p. 105).

Lead, dilute, PbTS. One millilitre contains 10 µg of lead.

Procedure. Dilute 10 ml of strong lead PbTS with sufficient water to produce 100 ml.

Note: Dilute lead PbTS must be freshly prepared.

Lead, strong, PbTS. One millilitre contains 100 µg of lead.

Procedure. Dissolve 0.1598 g of lead nitrate R in 5 ml of nitric acid (~1000 g/l) TS and sufficient water to produce 1000 ml.

Levamisole hydrochloride RS. International Chemical Reference Substance.

Levarterenol hydrogen tartrate R. $C_8H_{11}NO_3,C_4H_6O_6,H_2O$. Contains not more than 99% of $C_8H_{11}NO_3,C_4H_6O_6$, calculated with reference to the anhydrous substance.

Description. A white, or almost white, crystalline powder; odourless.

Solubility. Soluble in water; slightly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Specific optical rotation. Use a 50 mg/ml solution; $[\alpha]_D^{20\,°C} = -10$ to $-13\,°$.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the substance; not less than 45 mg/g and not more than 58 mg/g.

Assay. Dissolve about 0.4 g, accurately weighed, in glacial acetic acid R, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 ml/l) VS is equivalent to 31.93 mg of $C_8H_{11}NO_3,C_4H_6O_6$.

Levodopa RS. International Chemical Reference Substance.

Levonorgestrel RS. International Chemical Reference Substance.

Levothyroxine sodium RS. International Chemical Reference Substance.

Lidocaine RS. International Chemical Reference Substance.

Limulus amoebocyte lysate. Reconstitute the lysate as stated on the label. For each batch, confirm the stated sensitivity as prescribed under "Sensitivity of the lysate" in 3.4 Test for bacterial endotoxins. The sensitivity of the lysate is defined as the lowest concentration of endotoxin which yields a firm gel under test conditions and is expressed in endotoxin units per millilitre.

Lincomycin hydrochloride RS. International Chemical Reference Substance.

Lindane RS. International Chemical Reference Substance.

Liothyronine RS. International Chemical Reference Substance.

Lithium carbonate R. Li_2CO_3.
Description. A white, granular powder; odourless.
Solubility. Sparingly soluble in water; very slightly soluble in ethanol (~750 g/l) TS.

Lithium carbonate/trinitrophenol TS
Procedure. Dissolve 0.25 g of lithium carbonate R and 0.5 g of trinitrophenol R in sufficient water to produce 100 ml.

Lithium chloride (10 g/l) TS. A solution of lithium chloride R containing about 10 g of LiCl per litre.

Lithium chloride R. LiCl.
Description. White, deliquescent crystals or granules.
Solubility. Freely soluble in water; soluble in acetone R, ethanol (~750 g/l) TS, and ether R.
Storage. Store in a tightly closed container.

Lithium methoxide (0.1 mol/l) VS
Procedure. Dissolve 0.694 g of lithium R in 150 ml of methanol R and add sufficient toluene R to produce 1000 ml.
Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Dissolve about 0.15 g, accurately weighed, of benzoic acid R in 25 ml of dimethylformamide R and titrate with the lithium methoxide solution to a red end-point, using quinaldine red/methanol TS as indicator, as described under 2.6 Non-aqueous titration, Method B. Each 12.21 mg of benzoic acid is equivalent to 1 ml of lithium methoxide (0.1 mol/l) VS. Lithium methoxide solutions must be standardized immediately before use.

Lithium perchlorate R. $LiClO_4$.
Description. Small crystals.
Solubility. Freely soluble in water; sparingly soluble in ethanol (~750 g/l) TS, acetone R, ether R, and ethyl acetate R.

Lithium perchlorate/acetic acid TS
Procedure. Dissolve 10.64 g of lithium perchlorate R in sufficient glacial acetic acid R1 to produce 1000 ml.

Lithium R. Li.
Description. A soft metal whose freshly cut surface is silvery-grey, tarnishing rapidly in air.
Solubility. Reacts violently with water, yielding hydrogen and a solution of lithium hydroxide; soluble in methanol R, yielding hydrogen and a solution of lithium methoxide; practically insoluble in ether R.

Litmus paper R. (SRIP, 1963, p. 109).

Litmus R. (SRIP, 1963, p. 108).

Litmus TS
Procedure. Boil 10 g of litmus R with 40 ml of ethanol (~710 g/l) TS for 1 hour and pour away the clear liquid; repeat this operation twice with 30 ml of ethanol (~710 g/l) TS. Digest the washed litmus with 100 ml of water and filter.

Loperamide hydrochloride RS. International Chemical Reference Substance.

Macrogol 1000R
Description. A white, waxy mass.
Viscosity. At 100 °C, about 17.3 $mm^2 s^{-1}$.

Macrogol 200R
Description. A clear, colourless or almost colourless viscous liquid.
Solubility. Very soluble in acetone R and in ethanol (~750 g/l) TS; practically insoluble in ether R and in fatty oils.

Macrogol 200 TS
Procedure. Pour 500 ml of macrogol 200R into a 1000-ml, round-bottom flask. Evaporate any volatile components using a rotation evaporator. Heat to 60 °C and apply a vacuum with a pressure of 1.5–2.5 kPa for 6 hours.

Macrogol 20M R. Polyethylene glycol 20 000. A suitable grade to be used in gas-liquid chromatography.

Macrogol 400R. Polyethylene glycol 400. Macrogol 400R is a polymer of ethylene oxide and water, represented by the formula $H(OCH_2CH_2)_nOH$, in which the average value of *n* lies between 8.2 and 9.1.

Description. Clear colourless (or practically colourless) viscous liquid having a slight characteristic odour; slightly hygroscopic.

Average molecular weight. Transfer to a pressure flask 2.1 g of macrogol 400R, accurately weighed, and 25.0 ml of phthalic anhydride/pyridine TS. Insert the stopper in the flask, wrap the flask securely with cloth, and immerse it in a water-bath maintained at 96–100 °C, to the same depth as the mixture in the flask, for 1 hour. Remove the flask, retaining the cloth wrapping, and allow to cool in air to room temperature. To the contents of the flask add 50 ml of carbonate-free sodium hydroxide (0.5 mol/l) VS and 5 drops of phenolphthalein/pyridine TS. Titrate with carbonate-free sodium hydroxide (0.5 mol/l) VS to a pink end-point that remains for not less than 15 seconds. Perform a blank determination in a similar manner. Calculate the average molecular weight by multiplying by 4000 the weight, in g, of the test substance and dividing the result by the difference between the volume, in ml, of carbonate-free sodium hydroxide (0.5 mol/l) VS consumed for the test substance and the blank determination. The average molecular weight is between 380 and 420.

Mass density (ρ_{20}). 1.110–1.140 kg/l.

Congealing point. Between 4 and 8 °C, the congealing point being the average of 4 consecutive temperature readings, the highest and lowest of which differ by not more than 0.4 °C.

pH Value. Between 4.5 and 7.5, in a 50 g/l solution.

Acidity or alkalinity. Dissolve 5.0 g in 50 ml of water. Add a few drops of phenol red/ethanol TS. If the solution turns yellow, titrate with sodium hydroxide (0.01 mol/l) VS; if the solution turns red, titrate with hydrochloric acid (0.01 mol/l) VS. Not more than 2.0 ml of titrant should be required in either case.

Sulfated ash. Not more than 10 mg/g.

Heavy metals. Mix 4 g, accurately weighed, with 1 ml of hydrochloric acid (~70 g/l) TS and dilute with water to 25 ml. The limit is 50 µg/g.

Limit of monoethylene and diethylene glycols. Dissolve 50 g in 75 ml of diphenyl ether R in a 250-ml distillation flask. Slowly distil at a pressure of 100–250 Pa (1–2 mmHg) into a receiver that is graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate have been collected. Add 25.0 ml of water to the distillate, shake the receiving flask vigorously, and allow the layers to separate. Cool the container in an ice-bath to solidify and facilitate the removal of the layer of diphenyl ether R. Filter the water layer through filter-paper into a glass-stoppered, 50-ml graduated cylinder. To the filtrate add an equal volume of freshly distilled acetonitrile R, and shake the cylinder until solution is complete. Pipette 10 ml of the solution into 15 ml of ceric ammonium nitrate TS, mix, and within 2–5 minutes determine the absorbance of the resulting solution at about 525 nm. Use a blank consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of acetonitrile (400 g/l)

TS. Prepare a standard solution by mixing 10 ml of acetronile (400 g/l) TS, to which 30 mg of diethylene glycol R have been added, and 15 ml of ceric ammonium nitrate TS and determine the absorbance within 2–5 minutes at about 525 nm, using the same blank as above. The absorbance of the test solution should not exceed that of the standard solution.

Macrogol p-isooctylphenyl ether R. $C_{34}H_{62}O_{11}$, *p-tert*-octylphenoxy polyethoxy-ethanol. Use a suitable grade for the 3.2.2 Sterility testing of antibiotics.

Magnesium (0.1 mg/ml Mg) TS

Procedure. Dissolve 1.014 g of magnesium sulfate R in water, add 5 ml of sulfuric acid (~100 g/l) TS and dilute with water to 1000 ml.

Magnesium acetate R. $C_4H_6MgO_4,4H_2O$.

Description. Colourless crystals. Deliquescent.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS.

Magnesium chloride (0.1 mol/l) VS

Procedure. Dissolve 20.5 g of magnesium chloride R in sufficient water to produce 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution, carrying out the complexometric titration of magnesium under 2.5 Complexometric titrations using 25 ml of magnesium chloride solution. Each ml of disodium edetate (0.1 mol/l) VS is equivalent to 20.33 mg of $MgCl_2,6H_2O$.

Magnesium chloride R. $MgCl_2,6H_2O$ (SRIP, 1963, p. 110).

Magnesium oxide R. MgO.

Description. A white, very fine powder.

Solubility. Very slightly soluble in water; insoluble in ethanol (~750 g/l) TS.

Magnesium standard (10 µg/ml Mg) TS

Procedure. Dilute 10 ml of magnesium (0.1 mg/ml) TS with sufficient water to produce 100 ml.

Magnesium sulfate (50 g/l) TS. A solution of magnesium sulfate R containing about 50 g of $MgSO_4$ per litre.

Magnesium sulfate R. $MgSO_4,7H_2O$ (SRIP, 1963, p. 111).

Magnesium sulfate/sulfuric acid TS

Procedure. Dissolve 25 g of magnesium sulfate R in sufficient sulfuric acid (~100 g/l) TS to produce 100 ml.

Maleic acid R. $C_4H_4O_4$.
Description. Colourless crystals.
Melting temperature. About 135 °C.

Manganese dioxide R. MnO_2 (SRIP, 1963, p. 112).

Manganese sulfate (15 g/l) TS. Manganese sulfate R, dissolved in water to contain 15.0 g/l of $MnSO_4$.

Manganese sulfate R. $MnSO_4,H_2O$.
Description. Pale-red, slightly efflorescent crystals.
Solubility. Soluble in about 1 part of water and 0.6 part of boiling water; practically insoluble in ethanol (~750 g/l) TS.

Manganese/silver paper R
Procedure. To a mixture of equal volumes of silver nitrate (0.1 mol/l) VS and manganese sulfate (15 g/l) TS, add drop by drop sodium hydroxide (0.1 mol/l) VS until a persistent precipitate is produced, and filter. Soak strips of filter-paper (Whatman No. 1 is suitable) for 15 minutes in the solution, dry them at ambient temperature, protected from light and acidic or alkaline vapours. The manganese/silver paper R should be colourless.
Test for sensitivity. Place in a cylinder of about 40 ml capacity (height about 80 mm, internal diameter about 30 mm) 1.0 ml of ammonium chloride (10 µg/ml NH_4) TS. Add 9 ml of water and 1 g of magnesium oxide R. Immediately stopper the flask using a polyethylene cap below which a manganese/silver paper R is placed. Swirl the solution carefully so that magnesium particles do not come into contact with the reagent paper. Keep the cylinder at 50–60 °C for 1 hour. A true grey colour is produced on the reagent paper.

Mebendazole RS. International Chemical Reference Substance.

Medroxyprogesterone acetate RS. International Chemical Reference Substance.

Mefloquine hydrochloride RS. International Chemical Reference Substance.

Meglumine (100 g/l) TS. A solution of meglumine R containing about 100 g of $C_7H_{17}NO_5$ per litre.
Note: Meglumine (100 g/l) TS must be freshly prepared.

Meglumine R. $C_7H_{17}NO_5$. Meglumine as described in the monograph for "Meglumine".

Menadione R. 2-Methyl-1,4-naphthoquinone, $C_{11}H_8O_2$.
Description. Bright yellow crystals.
Melting temperature. About 106 °C.

Mercaptoacetic acid R
(thioglycolic acid R). $C_2H_4O_2S$ (SRIP, 1963, p. 206).

Mercuric acetate R. $C_4H_6HgO_4$ (SRIP, 1963, p. 112).

Mercuric acetate/acetic acid TS
Procedure. Dissolve 50 g of mercuric acetate R in sufficient glacial acetic acid R1, that has been neutralized, if necessary, to crystal violet/acetic acid TS with perchloric acid (0.1 mol/l) VS, to produce 1000 ml.

Mercuric bromide AsTS
Procedure. Dissolve 5 g of mercuric bromide R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Mercuric bromide paper AsR
Procedure. Use smooth, white filter-paper weighing 65–120 g/m^2. The thickness of the paper in mm should be approximately equal numerically to the weight expressed as above, divided by 400. Soak pieces of filter-paper, not less than 25 mm in width, in mercuric bromide AsTS, decant the superfluous liquid, suspend the paper over a non-metallic thread, and allow it to dry, protected from light.
Storage. Store the mercuric bromide paper AsR in stoppered bottles in the dark.
Note: Paper that has been exposed to sunlight or to vapours of ammonia must not be used as it produces only a pale stain or no stain at all.

Mercuric bromide R. $HgBr_2$ (SRIP, 1963, p. 113).

Mercuric chloride (2.7 g/l) TS. A solution of mercuric chloride R containing about 2.7 g of $HgCl_2$ per litre.

Mercuric chloride (65 g/l) TS. A solution of mercuric chloride R containing about 65 g of $HgCl_2$ per litre (approximately 0.25 mol/l).

Mercuric chloride R. $HgCl_2$ (SRIP, 1963, p. 113).

Mercuric chloride/ethanol TS
Procedure. Dissolve 2 g of mercuric chloride R in sufficient ethanol (~375 g/l) TS to produce 100 ml.

Mercuric iodide R. Mercury diiodide; HgI_2.
Description. A heavy, crystalline, scarlet-red powder; odourless.

Solubility. Slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS, acetone R, and ether R; soluble in solutions containing an excess of potassium iodide R.

Storage. Mercuric iodide R should be stored protected from light.

Mercuric nitrate (0.01 mol/l) VS

Procedure. Dissolve about 3.5 g, accurately weighed, of mercuric nitrate R in a mixture of 5 ml of nitric acid (~1000 g/l) TS and 500 ml of water, and dilute with water to 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.01 mol/l solution in the following manner: Transfer 20.0 ml to a conical flask, add 2 ml of nitric acid (~1000 g/l) TS and 2 ml of ferric ammonium sulfate (45 g/l) TS. Cool to below 20 °C, and titrate with ammonium thiocyanate (0.01 mol/l) VS to the first appearance of a permanent brownish colour.

Mercuric nitrate (0.02 mol/l) VS

Procedure. Weigh accurately about 6.85 g of mercuric nitrate R, dissolve in a mixture of 10 ml of nitric acid (~130 g/l) TS and 500 ml of water, and dilute with water to 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.02 mol/l solution following the method described under mercuric nitrate (0.01 mol/l) VS.

Mercuric nitrate R. $Hg(NO_3)_2,H_2O$.

Caution. Mercuric nitrate R is poisonous.

Description. A white or slightly yellow, deliquescent, crystalline powder.

Solubility. Soluble in water in the presence of a small quantity of nitric acid (~1000 g/l) TS.

Mercuric nitrate TS

Procedure. Dissolve 40 g of yellow mercuric oxide R in a mixture of 32 ml of nitric acid (~1000 g/l) TS and 15 ml of water.

Storage. Keep in a container protected from light.

Mercuric oxide, yellow, R. HgO (SRIP, 1963, p. 114).

Mercuric sulfate TS

Procedure. Mix 5 g of yellow mercuric oxide R with 40 ml of water and, while stirring, add 20 ml of sulfuric acid (~1760 g/l) TS, then add 40 ml of water and stir until completely dissolved.

Mercury R. Hg (SRIP, 1963, p. 115).

Mercury/nitric acid TS

Procedure. Dissolve 3 ml of mercury R in 27 ml of cold fuming nitric acid R and dilute the solution with an equal volume of water.

Storage. The solution should be stored, protected from light, and for not more than 2 months.

Metacycline hydrochloride RS. International Chemical Reference Substance.

Methanesulfonic acid R
Molecular formula. CH_4O_3S
Description. Colourless and corrosive liquid, strong irritant.
Solubility. Miscible with water.
Density (d). ~1.48.
Melting point. About 20 °C.

Methanol R. CH_3OH (SRIP, 1963, p. 117).

Methanol, dehydrated, R. Methanol R that complies with the following requirement: Water, not more than 1.0 mg/g.

Methenamine R. Hexamethylenetetramine, $C_6H_{12}N_4$. Contains not less than 99.0% of $C_6H_{12}N_4$.
Description. Colourless crystals or a crystalline powder; odourless.
Solubility. Soluble in water and ethanol (~750 g/l) TS.
Acidity and alkalinity. Dissolve 2.5 g in 25 ml of water. To 10 ml add 3 drops of phenolphthalein/ethanol TS; a pink colour is produced, which changes to red after the addition of 1 drop of carbonate-free sodium hydroxide (0.1 mol/l) VS. To a further 10 ml aliquot add 3 drops of bromothymol blue/ethanol TS; a blue colour is produced, which changes to green-blue after the addition of 3 drops of hydrochloric acid (0.1 mol/l) VS.
Sulfated ash. Not more than 0.5 mg/g.
Assay. Dissolve about 1.5 g, accurately weighed, in 10 ml of water, add 50 ml of sulfuric acid (0.5 mol/l) VS, and boil until the odour of formaldehyde is no longer perceptible. Titrate the excess of acid with sodium hydroxide (1 mol/l) VS, using methyl red/ethanol TS as indicator. Each ml of sulfuric acid (0.5 mol/l) VS is equivalent to 35.05 mg of $C_6H_{12}N_4$.

DL-Methionine RS. International Chemical Reference Substance.

Methotrexate RS. International Chemical Reference Substance.

Methyl green R. [α-[*p*-Dimethylamino)phenyl]-α-[4-(dimethyliminio)-2,5-cyclohexadien-1-ylidene]-*p*-tolyl]trimethylammonium dichloride; Basic blue 20; C.I. No. 42585; $C_{26}H_{33}Cl_2N_3$.
Description. A green powder.
Solubility. Soluble in water; soluble in sulfuric acid (~1760 g/l) TS giving a yellow solution and turning green on dilution.

Methyl green/iodomercurate paper R

Procedure. Dip strips of suitable filter-paper in a solution of 4 g of methyl green R in 100 ml of water and allow to dry in air. Then immerse the strips for 1 hour in a mixture composed of 14 g of potassium iodide R and 20 g of mercuric iodide R in 100 ml of water. Wash the strips with water until the washings are practically colourless and allow to dry in air.

Storage. Methyl green/iodomercurate paper R should be stored protected from light.

Methyl orange ethanol TS

Procedure. Dissolve 0.04 g of methyl orange R in sufficient ethanol (~150 g/l) TS to produce 100 ml.

Methyl orange R. Sodium salt of 4'-dimethylaminoazobenzene-4-sulfonic acid, $C_{14}H_{14}N_3NaO_3S$ (SRIP, 1963, p. 118).

Methyl orange/acetone TS. A saturated solution of methyl orange R in acetone R.

N-Methyl-N-nitrosotoluene-4-sulfonamide R. $C_8H_{10}N_2O_3S$.

Description. A yellow, crystalline powder.

Solubility. Insoluble in water; soluble in ethanol (~750 g/l) TS and ether R.

Melting temperature. About 60 °C.

N-Methylpiperazine R. $C_5H_{12}N_2$.

Mass density. ρ_{20} =0.902 kg/l.

Refractive index. n_D^{20} = 1.466.

Methyl red R. 4'-Dimethylaminoazobenzene-2-carboxylic acid, $C_{15}H_{15}N_3O_2$ (SRIP, 1963, p. 118).

Methyl red/ethanol TS

Procedure. Dissolve 25 mg of methyl red R in a mixture of 0.95 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~750 g/l) TS, warm the solution slightly and after cooling dilute with sufficient ethanol (~375 g/l) TS to produce 250 ml.

Methyl red/methylthioninium chloride TS

Procedure. Mix 20 ml of a 0.5 mg/ml solution of methyl red R in ethanol (~150 g/l) TS with 0.4 ml of a 20 mg/ml solution of methylthioninium chloride R in water.

Methyl silicone gum R. A suitable grade to be used in gas-liquid chromatography.

2-Methyl-5-nitroimidazole R. $C_4H_5N_3O_2$.
Melting temperature. About 253 °C.

Methyl violet 2B R. CI 42535; C.I. basic violet.
A commercially available reagent of suitable grade.
Melting point. About 137 °C, with decomposition.

Methylamine hydrochloride (20 g/l) TS. A solution of methylamine hydrochloride R containing about 20 g of CH_5N,HCl per litre.

Methylamine hydrochloride R. CH_5N,HCl.
Description. Deliquescent tetragonal tablets.
Solubility. Soluble in water and dehydrated ethanol R; practically insoluble in acetone R, ether R, and ethyl acetate R.
Melting point. About 228 °C.

Methyldopa RS. International Chemical Reference Substance.

Methylisobutylketone R. Isopropylacetone, $C_6H_{12}O$. (SRIP, 1963, p. 119).

Methyltestosterone RS. International Chemical Reference Substance.

Methylthioninium chloride (0.2 g/l) TS
Procedure. Dissolve 23 mg of methylthioninium chloride R in sufficient water to produce 100 ml.

Methylthioninium chloride (1 g/l) TS. A solution of methylthioninium chloride R containing about 1 g of $C_{16}H_{18}ClN_3S$ per litre.

Methylthioninium chloride R [methylene blue]. $C_{16}H_{18}ClN_3S,3H_2O$ (SRIP, 1963, p. 119).

Methylthymol blue mixture R
Procedure. Mix 1 part of methylthymol blue R with 100 parts of potassium nitrate R.

Methylthymol blue R. Tetrasodium [3*H*-2,1-benzoxathiol-3-ylidenebis[(6-hydroxy-5-isopropyl-2-methyl-*m*-phenylene)methylenenitrilo]]tetraacetic acid *S*,*S*-dioxide; $C_{37}H_{44}N_2Na_4O_{13}S$.
Description. A brownish-black powder.
Solubility. Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS.

Metronidazole benzoate RS. International Chemical Reference Substance.

Metoclopramide hydrochloride RS. International Chemical Reference Substance.

Metronidazole RS. International Chemical Reference Substance.

Miconazole nitrate RS. International Chemical Reference Substance.

Molybdenum trioxide R. MoO_3 (SRIP, 1963, p. 120).

Monoethanolamine (0.1 mol/l) VS. A solution of monoethanolamine R in water to contain 6.108 g of C_2H_7NO in 1000 ml.

Monoethanolamine R. C_2H_7NO.
Description. A clear, colourless to faintly yellow, viscous liquid; odour, ammoniacal.
Miscibility. Miscible with water, methanol R, and acetone R.
Boiling temperature. About 170 °C.
Mass density. ρ_{20} = 1.01 kg/l.
Refractive index, n_D^{20} = 1.453 – 1.455.

Mordant Black 11 indicator mixture R
Procedure. Mix 1 g of Mordant Black 11 R with 100 g of sodium chloride R.

Mordant Black 11 R
[eriochrome black R]. C.I. Mordant Black 11, C.I. No. 14645, Eriochrome Black T, Solochrome Black; sodium salt of 2-(2-hydroxy-6-nitro-4-sulfo-1-naphthylazo)-1-naphthol, $C_{20}H_{12}N_3NaO_7S$ (SRIP, 1963, p. 84).

Morpholine R. Tetrahydro-1,4-oxazine; C_4H_9NO (SRIP, 1963, p. 121).

N-(1-Naphthyl)ethylenediamine hydrochloride (1 g/l) TS. A solution of *N*-(1-naphthyl)ethylenediamine hydrochloride R containing about 1 g of $C_{12}H_{14}N_2$,2HCl per litre.

N-(1-Naphthyl)ethylenediamine hydrochloride (5 g/l) TS. A solution of *N*-(1-naphthyl)ethylenediamine hydrochloride R containing about 5 g of $C_{12}H_{14}N_2$,2HCl per litre.

N-(1-Naphthyl)ethylenediamine hydrochloride R. $C_{12}H_{14}N_2$,2HCl (SRIP, 1963, p. 124).

N-(1-Naphthyl)ethylenediamine hydrochloride/1-propanol TS
Procedure. To 7 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS add 3 ml of 1-propanol R.

N-(1-Naphthyl)ethylenediamine/ethanol TS
Procedure. Dissolve 5 g of *N*-(1-Naphthyl)ethylenediamine hydrochloride R in a mixture of equal volumes of ethanol (~750 g/l) TS and water to produce 1000 ml.

N-(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS
Procedure. Dissolve 0.1 g of *N*-(1-naphthyl)ethylenediamine hydrochloride R in 30 ml of water and dilute to 100 ml with propylene glycol R.
Note. N-(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS must be freshly prepared.

Naloxone hydrochloride RS. International Chemical Reference Substance.

Naphthalene-1,3-diol R. 1,3-Naphthalenediol; $C_{10}H_8O_2$.
Description. Colourless crystals.
Solubility. Freely soluble in water, ethanol (~750 g/l) TS, and ether R.
Melting temperature. About 124 °C.

Naphthalene-1,3-diol/ethanol TS
Procedure. Dissolve 0.2 g of naphthalene-1,3-diol R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

2-Naphthol R [β-naphthol R]. $C_{10}H_8O$ (SRIP, 1963, p. 122).

1-Naphthol R. $C_{10}H_8O$.
Description. Colourless crystals or a white, crystalline powder; odour, characteristic.
Solubility. Soluble in 5 parts of ethanol (~750 g/l) TS (may form a slightly opalescent, colourless or almost colourless solution).
Melting range. 93–96 °C.
Sulfated ash. Not more than 0.5 mg/g.

1-Naphthol TS1
Procedure. Dissolve 0.10 g of 1-naphthol R in 3 ml of sodium hydroxide (~150 g/l) TS and dilute with sufficient water to produce 100 ml.
Note: 1-Naphthol TS1 must be prepared immediately before use.

2-Naphthol TS1
Procedure. Dissolve 5 g of 2-naphthol R, freshly recrystallized, in 40 ml of sodium hydroxide (~80 g/l) TS and add sufficient water to produce 100 ml.
Note: 2-Naphthol TS1 must be freshly prepared.

1-Naphtholbenzein R. $C_{27}H_{20}O_3$.
Description. A reddish-brown powder.
Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l) TS, benzene R, ether R, and glacial acetic acid R.

1-Naphtholbenzein/acetic acid TS
Procedure. Dissolve 0.2 g of 1-naphtholbenzein R in sufficient glacial acetic acid R to produce 100 ml.

1-Naphthol/ethanol TS
Procedure. Dissolve 0.05 g of 1-naphthol R in 60 ml of ethanol (~750 g/l) TS and add sufficient water to produce 100 ml.

Neamine hydrochloride RS. International Chemical Reference Substance.

Nelfinavir mesilate RS. International Chemical Reference Substance.

Neostigmine metilsulfate RS. International Chemical Reference Substance.

Neutral red R. C.I. 50040; C.I. Basic Red; $C_{15}H_{17}ClN_4$ (SRIP, 1963, p. 124).

Neutral red/ethanol TS
Procedure. Dissolve 0.1 g of neutral red R in sufficient ethanol (~375 g/l) TS to produce 100 ml.

Nevirapine, anhydrous RS. International Chemical Reference Substance.

Nevirapine impurity B RS. International Chemical Reference Substance.

Niclosamide RS. International Chemical Reference Substance.

Nicotinamide RS. International Chemical Reference Substance.

Nicotinic acid RS. International Chemical Reference Substance.

Nifedipine RS. International Chemical Reference Substance.

Nifurtimox RS. International Chemical Reference Substance.

Niridazole RS. International Chemical Reference Substance.

Niridazole-chlorethylcarboxamide RS. International Chemical Reference Substance.

Nitric acid (~1000 g/l) TS [nitric acid (70 per cent.) R]. (SRIP, 1963, p. 125); *d* ~ 1.41.

Nitric acid (~130 g/l) TS
Procedure. Dilute 130 ml of nitric acid (~1000 g/l) TS with sufficient water to produce 1000 ml (approximately 2 mol/l); *d* ~ 1.07.

Nitric acid (0.05 mol/l) VS. Nitric acid (~1000 g/l) TS, diluted with water to contain 3.151 g of HNO_3 in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution by following the method described under nitric acid (1 mol/l) VS.

Nitric acid (1 mol/l) VS. Nitric acid (~1000 g/l) TS, diluted with water to contain 63.10 g of HNO_3 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: Dissolve 2 g of anhydrous sodium carbonate R in 50 ml of water and titrate with the nitric acid solution using 0.1 ml of methyl orange/ethanol TS as indicator until the solution just becomes reddish yellow. Boil for 2 minutes; the solution reverts to yellow. Cool and continue the titration until the reddish yellow colour is restored. Each ml of nitric acid (1 mol/l) VS is equivalent to 0.0530 g of Na_2CO_3.

Nitric acid (15 g/l) TS. Nitric acid (~1000 g/l) TS, diluted with water to contain 15.0 g/l of HNO_3.

Nitric acid (3 g/l) TS. Nitric acid (~1000 g/l) TS, diluted with water to contain 3.0 g/l of HNO_3.

Nitric acid, fuming, R. HNO_3 (SRIP, 1963, p. 126); $d \sim 1.5$.

4-Nitroaniline R [*p*-nitroaniline R]. $C_6H_6N_2O_2$ (SRIP, 1963, p. 127).

4-Nitroaniline TS1

Procedure. Dissolve 5 g of 4-nitroaniline R in sufficient hydrochloric acid (1 mol/l) VS to produce 1000 ml.

4-Nitroaniline TS2

Procedure. Dissolve 0.4 g of 4-nitroaniline R in 60 ml of hydrochloric acid (1 mol/l) VS, cool to 15 °C, and add sufficient sodium nitrite (100 g/l) TS until 1 drop of the mixture turns starch/iodine paper R blue.

Note: 4-Nitroaniline TS2 must be freshly prepared.

Nitrobenzene R. $C_6H_5NO_2$ (SRIP, 1963, p. 128).

4-Nitrobenzoyl chloride R [*p*-nitrobenzoyl chloride R]. $C_7H_4ClNO_3$ (SRIP, 1963, p. 128).

Nitrofurantoin RS. International Chemical Reference Substance.

Nitrogen monoxide R. NO.

Nitric oxide, washed with water.

A commercially available gas of suitable grade.

Nitrogen monoxide and nitrogen dioxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for an oxidizing layer Cr(VI) salt and the diphenyl-benzidine indicator. The minimum value indicated is 5 µl/l or less, with a relative standard deviation

of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Nitrogen R. N_2 (SRIP, 1963, p. 129).

Nitrogen for chromatography R.
Contains not less than 99.95% v/v of N_2.

Nitrogen, oxygen-free, R. Nitrogen R which has been freed from oxygen by passing it through alkaline pyrogallol TS.

Nitromethane R. CH_3NO_2.
Description. A colourless, oily liquid.
Miscibility. Miscible with water, ethanol (~750 g/l) TS, ether R, and di-methylformamide R.
Mass density. ρ_{20} = about 1.13 kg/l.
Refractive index. n_D^{20} = about 1.380.
Boiling temperature. About 101 °C.

Nitrophenanthroline R. 5-Nitro-1,10-phenanthroline; $C_{12}H_7N_3O_2$.
Description. A white powder; odourless.
Solubility. Soluble in water.
Melting range. 198–200 °C.

Nitrophenanthroline TS
Procedure. Dissolve 0.15 g of nitrophenanthroline R in 15 ml of freshly prepared ferrous sulfate (7 g/l) TS.

1-Nitroso-2-naphthol-3,6-disodium disulfonate (2 g/l) TS. A solution of 1-nitroso-2-naphthol-3,6-disodium disulfonate R containing about 2 g of $C_{10}H_5NNa_2O_8S_2$ per litre.

1-Nitroso-2-naphthol-3,6-disodium disulfonate R [1-nitroso-2-naphthol-3,6-disodium sulfonate R]. $C_{10}H_5NNa_2O_8S_2$ (SRIP, 1963, p. 129).

Nonoxinol 9 RS. International Chemical Reference Substance.

Norethisterone acetate RS. International Chemical Reference Substance.

Norethisterone enantate RS. International Chemical Reference Substance.

Norethisterone RS. International Chemical Reference Substance.

Noscapine RS. International Chemical Reference Substance.

Nystatin RS. International Chemical Reference Substance.

Octanoic acid R. Caprylic acid, $C_8H_{16}O_2$.
Description. A colourless, oily liquid.
Boiling temperature. About 237 °C.
Mass density. ρ_{20} = about 0.92 kg/l.

Oil detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for the sulfuric acid indicator. The minimum value indicated is 0.1 mg/m^3, with a relative standard deviation of at most ±30%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Olive oil R. A commercially available reagent of suitable grade.

Opalescence standard TS1
Procedure. Dilute 15 ml of Opalescence stock standard TS with sufficient water to produce 1000 ml.
Shelf-life. Use within 24 hours after preparation.

Opalescence standard TS2
Procedure. Dilute 5.0 ml of Opalescence standard TS1 with sufficient water to produce 100 ml. Mix well and shake before use.
Note: Opalescence standard TS2 must be freshly prepared.

Opalescence standard TS3
Procedure. Dilute 10 ml of opalescence standard TS1 with sufficient water to produce 100 ml. Mix well and shake before use.
Note: Opalescence standard TS3 must be freshly prepared.

Opalescence stock standard TS
Procedure. Dissolve 1.0 g of hydrazine sulfate R in sufficient water to produce 100 ml and allow to stand for 4–6 hours. To 25.0 ml of this solution add a solution of 2.5 g of methenamine R dissolved in 25.0 ml of water, mix well, and allow to stand for 24 hours.
Storage. Store in a glass container free from surface defects.
Shelf-life. Use within 2 months after preparation.

Oracet blue B R. Solvent blue 19; a mixture of 1-methylamino-4-anilinoanthraquinone ($C_{21}H_{16}N_2O_2$) and 1-amino-4-anilinoanthraquinine ($C_{20}H_{14}N_2O_2$).

Oracet blue B/acetic acid TS
Procedure. Dissolve 0.5 g of oracet blue B R in sufficient glacial acetic acid R1 to produce 100 ml.

Osmium tetroxide R. OsO_4.

Caution. The fumes are corrosive to the eyes, the mucous membranes, and the skin.

Description. Yellow, needle-shaped crystals or a yellow, crystalline mass; hygroscopic; light sensitive; odour, pungent.

Solubility. Soluble in water, ethanol (~750 g/l) TS, and ether R.

Ox brain, acetone-dried, R

Procedure. Cut into small pieces a fresh ox brain previously freed from vascular and connective tissue. Place in acetone R for preliminary dehydration. Complete the dehydration by pounding in a mortar 30 g of the material with successive quantities, each of 75 ml of acetone R, until a dry powder is obtained after filtration. Dry at 37 °C for 2 hours or until the odour of acetone is no longer perceptible.

Oxalic acid (0.05 g/l) TS. A solution of oxalic acid R containing 0.05 g of $C_2H_2O_4$ in 1000 ml.

Procedure. Dissolve 0.07 g of oxalic acid R in sufficient water to produce 1000 ml.

Oxalic acid R. $C_2H_2O_4,2H_2O$ (SRIP, 1963, p. 131).

Oxalic acid/sulfuric acid TS

Procedure. Dissolve 5 g of oxalic acid R in a sufficient quantity of a cooled mixture of equal volumes of sulfuric acid (~1760 g/l) TS and water to produce 100 ml.

Oxamniquine RS. International Chemical Reference Substance.

Oxytetracycline dihydrate RS. International Chemical Reference Substance.

Oxytetracycline hydrochloride RS. International Chemical Reference Substance.

Pancreatic digest of casein R. (SRIP, 1963, p. 132).

Papaic digest of soybean meal R. (SRIP, 1963, p. 134).

Papaverine hydrochloride RS. International Chemical Reference Substance.

Paracetamol R. Paracetamol as described in the monograph for "Paracetamol".

Paracetamol, 4-aminophenol-free, R. Paracetamol as described in the monograph for "Paracetamol", or paracetamol recrystallized from water until it complies with the following test:

Dissolve 5 g of the dried material in a mixture of equal volumes of methanol R and water and dilute to 100 ml with this solvent mixture. Add 1.0 ml of alkaline sodium nitroprusside TS, mix, and allow to stand for 30 minutes; no blue or green colour is produced.

Paraffin, liquid, R. (SRIP, 1963, p. 135).

Paraformaldehyde R. $(CH_2O)_n$.
Description. A white, crystalline powder; odour, characteristic of formaldehyde.
Solubility. Slowly soluble in cold water, freely soluble in hot water, with evolution of formaldehyde; practically insoluble in ethanol (~750 g/l) TS and ether R.
Solubility in ammonia. Dissolve 1 g in 10 ml of ammonia (~100 g/l) TS; a practically clear and colourless solution is produced.
Sulfated ash. Not more than 1.0 mg/g.
Acidity or alkalinity. Shake 1 g with 20 ml of water for 1 minute and filter; the nitrate is neutral to litmus paper R.

Paraldehyde R. 2,4,6-Trimethyl-*s*-trioxane; $C_6H_{12}O_3$.
Description. Liquid; odour, characteristic, aromatic.
Boiling point. About 124 °C.
Mass density. $\rho_{20} = 0.994$ kg/l.

Paromomycin sulfate RS. International Chemical Reference Substance.

Penicillinase R. An enzyme, usually obtained from culture filtrates of a strain of *Bacillus cereus,* which has the specific property of inactivating penicillin by splitting the bond linking the nitrogen of the thiazolidine to the adjacent carbonyl carbon and thus releasing a carboxyl group. It is precipitated from solutions in water by acetone R, ethanol (~750 g/l) TS, and dioxan R, but inactivated by several hours' contact with these solvents; it is rapidly inactivated by ethyl acetate R. In place of penicillinase R a sterile filtrate obtained by fermentation of a penicillinase-producing organism in a suitable medium, described below under "Preparation of penicillinase", may also be used directly.
Description. Small, brown, easily pulverizable pieces or granules.
Solubility. Freely soluble in water, forming a slightly opalescent solution.
Preparation of penicillinase. Dissolve 10 g of pancreatic digest of casein R, 2.7 g of potassium dihydrogen phosphate R, and 5.9 g of sodium citrate R in 200 ml of water, adjust the alkalinity to pH 7.2 with sodium hydroxide (~200 g/l) TS, and dilute to 1000 ml with water. Dissolve 0.4 g of magnesium sulfate R in 5 ml of water and add 1 ml of ferrous ammonium sulfate (1 g/l) TS and sufficient water to produce 10 ml. Sterilize both solutions by heating in an autoclave, cool, mix, distribute in shallow layers in conical flasks and inoculate with a suitable strain (*Bacillus cereus*, NCTC 9946, is suitable). Allow the flasks to stand at 18–37 °C until growth is apparent and then

maintain at 35–37 °C for 16 hours, shaking constantly to ensure maximum aeration. Centrifuge and sterilize the supernatant liquid by filtration through a suitable membrane filter.

Penicillinase TS. A sterile aqueous solution of penicillinase R. To test the activity of penicillinase TS, carry out the "Penicillinase assay". The time required for iodine decolorization is not more than 36 seconds.

Penicillinase assay. Carry out the assay in test-tubes of borosilicate glass, 15 cm long and about 20 mm in internal diameter, immersed in a water-bath of 30 ± 1 °C. All reagents should have a temperature of 30 °C before use.

To the test-tube add the reagents in the following order: 1.6 ml of gelatin TS. 0.4 ml of penicillinase TS to be tested, 1 drop of starch TS, and 1 ml of benzylpenicillin sodium TS, blowing out the last reagent from a 1-ml pipette. Start the stop-watch and after 15 seconds add 2.0 ml of iodine (0.01 mol/l) VS, recording the time of decolorization of iodine from the time of the addition of benzylpenicillin sodium TS. The activity of penicillinase TS is calculated from the results of the assay. The time of decolorization of strictly 36 seconds corresponds to a penicillinase activity equivalent to a rate of decomposition (at 30 °C at pH 7.0) of 220 mg of benzylpenicillin sodium R per hour per ml of penicillinase TS.

Storage. Store between 0 and 2 °C and use within 2–3 days. When dried from the frozen state and kept in sealed ampoules, penicillinase TS may be stored for several months.

Pentamidine isetionate RS. International Chemical Reference Substance.

n-Pentane R. C_5H_{12}.

A commercially available reagent of suitable grade.

Description. A colourless, volatile liquid.

Boiling point. About 36 °C.

Relative density. d_D^{20} = about 1.359.

Transmittance. Not less than 20% at 200 nm, 50% at 210 nm, 85% at 220 nm, 93% at 230 nm, and 98% at 240 nm, determined using water in the reference cell.

1-Pentanesulfonic acid sodium salt R. $C_5H_{11}NaO_3S,H_2O$.

Description. A white, crystalline powder.

Solubility. Soluble in water.

Clarity and colour of solution. A solution of 1 g in 25 ml of water is clear and colourless.

Water. Determined by the Karl Fischer method; not more than 20 mg/g.

1-Pentanesulfonic acid TS

Procedure. Dissolve 0.960 g of 1-pentanesulfonic acid sodium salt R in 1000 ml of deaerated acetic acid (5.0 g/l) TS and adjust the pH to 4.3 with ammonia (~260 g/l) TS. Filter and deaerate before use.

Peptone (1 g/l) TS1

Procedure. Dissolve 1.0 g of peptone R1 (or similar peptic digest of animal tissue) in sufficient water to produce 1000 ml, filter or centrifuge to clarify, adjust the pH to 7.1 ± 0.2, place 100 ml portions into individual vessels, and sterilize by maintaining at 121 °C for 18–20 minutes.

Peptone (1 g/l) TS2

Procedure. Dissolve in water, while heating, 1.0 g of dried peptone R and 9 g of sodium chloride R and dilute with sufficient water to produce 1000 ml. Adjust to pH 8.0–8.4 and boil for 20 minutes. Filter, adjust to pH 7.2–7.4, and sterilize by maintaining at 115 °C for 30 minutes.

Peptone (5 g/l) TS

Procedure. Dissolve in water, while heating, 5.0 g of dried peptone R and 7 g of sodium chloride R and dilute with sufficient water to produce 1000 ml. Adjust to pH 8.0–8.4 and boil for 20 minutes. Filter, adjust to pH 7.2–7.4, and sterilize by maintaining at 115 °C for 30 minutes.

Peptone R1. Dried peptone R that conforms to the following requirement: An autoclaved solution containing 0.02 g/ml is clear and neutral or almost neutral.

Peptone, dried, R. (SRIP, 1963, p. 137).

Perchloric acid (~1170 g/l) TS [perchloric acid (70 per cent, w/w) R]. (SRIP, 1963, p. 137); $d \sim 1.67$.

Perchloric acid (~140 g/l) TS. Perchloric acid (~1170 g/l) TS, diluted with water to contain 141 g/l of $HClO_4$; $d \sim 1.09$.

Perchloric acid (0.02 mol/l) VS

Procedure. Dilute 20 ml of perchloric acid (0.1 mol/l) VS with sufficient glacial acetic acid R1 to produce 100 ml.

Water and method of standardization. Immediately before use determine the content of water and ascertain the exact concentration of the solution following the methods described under perchloric acid (0.1 mol/l) VS.

Perchloric acid (0.05 mol/l) VS

Procedure. To 900 ml of glacial acetic acid R1 at about 25 °C, add 4.2 ml of perchloric acid (~1170 g/l) TS, mix, add 15 ml of acetic anhydride R, and mix again. Cool to room temperature, add sufficient glacial acetic acid R1 to produce 1000 ml, and allow to stand for 24 hours.

Water and method of standardization. Determine the content of water and ascertain the exact concentration of the solution following the method described under perchloric acid (0.1 mol/l) VS.

Perchloric acid (0.1 mol/l) VS

Procedure. To 900 ml of glacial acetic acid R1, at about 25 °C, add 8.2 ml of perchloric acid (~1170 g/l) TS, mix, add 32 ml of acetic anhydride R, and mix again. Cool to room temperature, add sufficient glacial acetic acid R1, to produce 1000 ml, and allow to stand for 24 hours.

Water. Determine the content by the Karl Fischer method. If necessary, add sufficient water or acetic anhydride R to adjust the content of water to between 0.1 and 2.0 mg/ml, and allow to stand for a further 24 hours.

Method of standardization. Ascertain the exact concentration by titrating 0.5 g, accurately weighed, of potassium hydrogen phthalate R, previously dried at 120 °C for 2 hours, using method A, as described under 2.6 Non-aqueous titration. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.42 mg of $C_8H_5KO_4$. Record the temperature at which the standardization is carried out.

Perchloric acid TS

Procedure. Dilute 82 ml of perchloric acid (~1170 g/l) TS with sufficient water to produce 1000 ml (approximately 1 mol/l).

Perchloric acid/dioxan (0.1 mol/l) VS

Procedure. Mix 8.5 ml of perchloric acid (~1170 g/l) TS with sufficient dioxan R, which has been especially purified by adsorption, to produce 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution by titrating 0.7 g of potassium hydrogen phthalate R, accurately weighed and previously dried at 120 °C for 2 hours, using Method A, as described under 2.6 Non-aqueous titration. Each ml of perchloric acid/dioxan (0.1 mol/l) VS is equivalent to 20.42 mg of $C_8H_5KO_4$.

Periodic-acetic acid TS

Procedure. Dissolve 0.446 g of sodium metaperiodate R in 2.5 ml of sulfuric acid (~570 g/l) TS and dilute to 100 ml with glacial acetic acid R.

Petroleum, light, R [light petroleum R]. (SRIP, 1963, p. 108).

Petroleum, light, R1

Description. A colourless, very volatile, highly inflammable liquid.

Boiling range. 40–60 °C.

Mass density. ρ_{20} = 0.630–0.650 kg/l.

o-Phenanthroline (1 g/l) TS

Procedure. Dissolve 0.11 g of o-phenanthroline R in sufficient water to produce 100 ml.

o-Phenanthroline R. 1,10-Phenanthroline, $C_{12}H_8N_2,H_2O$ (SRIP, 1963, p. 138).

o-Phenanthroline TS

Procedure. Dissolve 0.7 g of ferrous sulfate R in about 70 ml of water, add about 1.5 g of o-phenanthroline R and sufficient water to produce 100 ml.

Phenobarbital RS. International Chemical Reference Substance.

Phenol (50 g/l) TS. A solution of phenol R containing about 50 g of C_6H_6O per litre.

Phenol R. C_6H_6O.

Description. Colourless, or at most faintly pink, cohering or separate acicular crystals, or crystalline masses; odour, characteristic. Corrosive, and blanches the skin and mucous membranes.

Solubility. Soluble in about 15 parts of water and in about 100 parts of liquid paraffin R; freely soluble in ethanol (~750 g/l) TS, and ether R.

Completeness of solution. 1.0 g dissolves completely in 15 ml of water at 15 °C.

Congealing temperature. Not below 40.5 °C.

Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; leaves not more than 0.5 mg/g of residue.

Phenol red R. Phenolsulfonphthalein, $C_{19}H_{14}O_5S$ (SRIP, 1963, p. 139).

Phenol red/ethanol TS

Procedure. Dissolve 0.05 g of phenol red R in a mixture of 2.85 ml of sodium hydroxide (0.05 mol/l) VS and 5 ml of ethanol (~710 g/l) TS. Warm the solution slightly and after cooling dilute with sufficient ethanol (~150 g/l) TS to produce 250 ml.

Phenoldisulfonic acid (250 g/l) TS. A commercially available reagent of suitable grade.

Phenoldisulfonic acid TS

Description. A clear liquid which may develop a pale brown colour on storage.

Procedure. Either of the following methods of preparation can be used: (1) To 3 g of phenol R add 20 ml of sulfuric acid (~1760 g/l) TS and heat on a water-bath for 6 hours; store in a stoppered vessel. (2) Dilute phenoldisulfonic acid (250 g/l) TS with sulfuric acid (~1760 g/l) TS to contain 150 g of phenol per litre.

Sensitivity to nitrate. Evaporate a solution containing 0.1 mg of potassium nitrate R to dryness in a porcelain dish on a water-bath. Cool, add 1.0 ml of the solution to be examined, and allow to stand for 10 minutes. Add 10 ml of water, cool, add 10 ml of ammonia (~100 g/l) TS, and dilute to 25 ml with water; a distinct yellow colour is produced when compared with a solution prepared similarly but omitting the potassium nitrate R.

Phenolphthalein R. $C_{20}H_{14}O_4$ (SRIP, 1963, p. 139).

Phenolphthalein/ethanol TS
Procedure. Dissolve 1.0 g of phenolphthalein R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Phenolphthalein/pyridine TS
Procedure. Dissolve 1.0 g of phenolphthalein R in sufficient pyridine R to produce 100 ml.

Phenoxyacetic acid R. Phenoxyethanoic acid; $C_8H_8O_3$.
Description. White or almost white crystals.
Melting temperature. About 98 °C.

2-Phenoxyethanol R. $C_8H_{10}O_2$.
Description. A clear, colourless, oily liquid; odour, faintly aromatic.
Miscibility. Slightly miscible with water; freely miscible with ethanol (~750 g/l) TS and ether R.
Mass density. ρ_{20} = about 1.1 kg/l.
Refractive index. n_D^{20} = about 1.537.
Freezing point. Not below 12.0 °C.

Phenoxymethylpenicillin calcium RS. International Chemical Reference Substance.

Phenoxymethylpenicillin potassium RS. International Chemical Reference Substance.

Phenoxymethylpenicillin RS. International Chemical Reference Substance.

1,4-Phenylenediamine dihydrochloride R. $C_6H_8N_2,2HCl$.
Description. A white to pale tan, crystalline powder, turning pink on exposure to air.
Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS and ether R.
Storage. Keep in a well-closed container, protected from light.

2-Phenylethanol R. $C_8H_{10}O$. A suitable grade to be used in gas-liquid chromatography.

2-Phenylethanol TS
Procedure. Dissolve 1 g of 2-phenylethanol R in sufficient methanol R to produce 50 ml.

Phenylhydrazine hydrochloride (10 g/l) TS. A solution of phenylhydrazine hydrochloride R containing 10 g of $C_6H_8N_2,HCl$ in 1000 ml.

Phenylhydrazine hydrochloride R. $C_6H_8N_2,HCl$ (SRIP, 1963, p. 140).

Phenylhydrazine R. $C_6H_8N_2$ (SRIP, 1963, p. 140).

Phenylhydrazine/hydrochloric acid TS
Procedure. Dissolve 0.75 g of phenylhydrazine hydrochloride R in 50 ml of water and shake with 2 g of charcoal R. Filter, add 25 ml of hydrochloric acid (~420 g/l) TS and sufficient water to produce 200 ml.

Phenylhydrazine/sulfuric acid TS
Procedure. Dissolve 65 mg of phenylhydrazine hydrochloride R, previously recrystallized from ethanol (~710 g/l) TS, in a sufficient quantity of a mixture of 170 ml of sulfuric acid (~1760 g/l) TS and 80 ml of water to produce 100 ml.
Note: Phenylhydrazine/sulfuric acid TS must be freshly prepared.

Phenyl/methylpolysiloxane R. A suitable grade of a mixture to be used in gas chromatography composed of 5 g of phenylpolysiloxane and 95 g of methylpolysiloxane per 100 g.

Phenytoin RS. International Chemical Reference Substance.

pH-Indicator paper R. A paper impregnated with a suitable mixture of colour indicators such that the changes in colour permit estimation of the pH of a solution with an adequate sensitivity (usually 1 pH unit), at least in the pH range 1–10.

Phloroglucinol R. Benzene-1,3,5-triol dihydrate; $C_6H_6O_3,2H_2O$.
Description. White or pale cream coloured crystals.
Melting point. About 220 °C.

Phosphate buffer, pH 4.0, TS
Procedure. Dissolve 5.04 g of disodium hydrogen phosphate R and 3.01 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml and adjust the pH to 4.0 with glacial acetic acid R.

Phosphate buffer, pH 6.4, TS
Procedure. Dissolve 1.36 g of potassium dihydrogen phosphate R in 50 ml of carbon-dioxide-free water R, add 12.60 ml of carbonate-free sodium hydroxide (0.2 mol/l) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 ml.

Phosphate buffer, pH 6.9, TS
Procedure. Dissolve 3.40 g of potassium dihydrogen phosphate R and 3.55 g of disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Phosphate buffer, pH 7.0 (0.067 mol/l), TS

Procedure. Dissolve 0.908 g of potassium dihydrogen phosphate R in sufficient water to produce 100 ml. Separately dissolve 2.38 g of disodium hydrogen phosphate R in sufficient water to produce 100 ml. Mix 38.9 ml of the potassium phosphate solution with 61.1 ml of the sodium phosphate solution.

Phosphate buffer, pH 7.0, TS

Procedure. Dissolve 5.76 g of anhydrous disodium hydrogen phosphate R and 3.55 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml.

Phosphate buffer, pH 7.2, TS

Procedure. Dissolve 6.80 g of potassium dihydrogen phosphate R and 1.40 g of sodium hydroxide R in sufficient water to produce 1000 ml.

Phosphate buffer, pH 7.4, TS

Procedure. Dissolve 6.8 g of potassium dihydrogen phosphate R in 250 ml of water and add 393.4 ml of sodium hydroxide (0.1 mol/l) VS.

Phosphate buffer, pH 7.6, TS

Procedure. Place 1.36 g of potassium dihydrogen phosphate R in a 200-ml volumetric flask, dissolve it in water, add 42.4 ml of sodium hydroxide (0.2 mol/l) VS, and dilute to volume with water.

Phosphate buffer, pH 8.0, TS

Procedure. Dissolve 8.95 g of anhydrous disodium hydrogen phosphate R and 0.50 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml.

Phosphate buffer, sterile[1], pH 10.5, TS1

Procedure. Dissolve 35.0 g of dipotassium hydrogen phosphate R in water, add 20 ml of sodium hydroxide (1 mol/l) VS and sufficient water to produce 1000 ml. If necessary, adjust the pH to 10.5 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 4.5, TS

Procedure. Dissolve 13.6 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 4.45–4.55 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

[1] The adjustment of the pH, if necessary, should be effected before sterilization of the solution.

Phosphate buffer, sterile[1], pH 6.0, TS1

Procedure. Dissolve 2.0 g of dipotassium hydrogen phosphate R and 8.0 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 6.0, TS2

Procedure. Dissolve 1.16 g of anhydrous disodium hydrogen phosphate R and 7.96 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 6.0, TS3

Procedure. Dissolve 20.0 g of dipotassium hydrogen phosphate R and 80.0 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 5.95–6.05 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 7.0, TS

Procedure. Dissolve 5.76 g of anhydrous disodium hydrogen phosphate R and 3.55 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 6.95–7.05 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 7.2, TS

Procedure. Dissolve 6.80 g of potassium dihydrogen phosphate R and 1.4 g of sodium hydroxide R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 7.1–7.3 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 7.8, TS

Procedure. Dissolve 6.8 g of potassium dihydrogen phosphate R in water, add 45.2 ml of sodium hydroxide (1 mol/l) VS and sufficient water to produce 1000 ml. If necessary, adjust the pH to 7.8 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

[1] The adjustment of the pH, if necessary, should be effected before sterilization of the solution.

Phosphate buffer, sterile[1], pH 8.0, TS1

Procedure. Dissolve 16.73 g of dipotassium hydrogen phosphate R and 0.52 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 7.9–8.1 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate buffer, sterile[1], pH 8.0, TS2

Procedure. Dissolve 8.95 g of anhydrous disodium hydrogen phosphate R and 0.50 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. If necessary, adjust the pH to 7.9–8.1 with phosphoric acid (~1440 g/l) TS or potassium hydroxide (~110 g/l) TS and then sterilize the solution for 20 minutes in an autoclave at 120 °C.

Phosphate standard (5 µg/ml) TS

Procedure. Dissolve 0.716 g of potassium dihydrogen phosphate R in sufficient water to produce 1000 ml. Immediately before use dilute 1 ml to 100 ml with water.

Phosphate standard buffer, pH 6.8, TS

Procedure. Dissolve 3.40 g of potassium dihydrogen phosphate R and 3.53 g of anhydrous disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Phosphate standard buffer, pH 7.4, TS

Procedure. Dissolve 1.18 g of potassium dihydrogen phosphate R and 4.30 g of anhydrous disodium hydrogen phosphate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Phosphate/citrate buffer pH 4.5, TS

Procedure. Dissolve 2.15 g of disodium hydrogen phosphate R in 30 ml of water and adjust the pH of the solution to 4.5 with citric acid (20 g/l) TS.

Phosphate/citrate buffer pH 6.0, TS

Procedure. Dissolve 4.52 g of disodium hydrogen phosphate R in 60 ml of water, add 35 ml of citric acid (20 g/l) TS, and, if necessary, adjust the pH of the solution to 6.0.

Phosphomolybdic acid (80 g/l) TS. A solution of phosphomolybdic acid R containing about 100 g of $H_3PO_4,12MoO_3,24H_2O$ per litre.

Phosphomolybdic acid R. $H_3PO_4,12MoO_3,24H_2O$ (SRIP, 1963, p. 141).

[1] The adjustment of the pH, if necessary, should be effected before sterilization of the solution.

Phosphomolybdic acid/ethanol TS
Procedure. Dissolve 5 g of phosphomolybdic acid R in sufficient dehydrated ethanol R to produce 100 ml.

Phosphoric acid (~105 g/l) TS
Procedure. Mix about 115 g of phosphoric acid (~1440 g/l) TS with 885 g of water.

Phosphoric acid (~1440 g/l) TS [phosphoric acid R]. (SRIP, 1963, p. 141); *d* ~ 1.7.

Phosphoric acid (~20 g/l) TS
Procedure. To 23 g of phosphoric acid (~1440 g/l) TS add 987 g of water and mix.

Phosphoric acid (~2.8 g/l) TS
Procedure. Dilute 2 ml of phosphoric acid (~1440 g/l) TS with sufficient water to produce 100 ml.

Phosphorus pentoxide R. P_2O_5 (SRIP, 1963, p. 142).

Phosphorus, red R
Description. A dark red powder.
Solubility. Insoluble in water and dilute acids.
Soluble matter. Heat 2.0 g with 30 ml of acetic acid (~300 g/l) TS on a water-bath for 15 minutes, cool, dilute to 50 ml, filter, evaporate 25 ml of the filtrate on a water-bath and dry at 110 °C for 2 hours; the residue weighs not more than 50 mg.
Yellow phosphorus. Shake 5.0 g with 20 ml of carbon disulfide R in a glass-stoppered cylinder, filter, and immerse in the filtrate a strip of filter-paper, 10 cm by 0.5 cm, previously immersed in copper(II) sulfate (80 g/l) TS, and allow to dry in the air; no stain is produced.
Loss on drying. Dry to constant weight over sulfuric acid (~1760 g/l) TS; it loses not more than 10 mg/g.

Phosphotungstic acid TS
Procedure. Dissolve 25 g of sodium tungstate R in 175 ml of water and add 18.75 ml of phosphoric acid (~1440 g/l) TS. Heat under a reflux condenser for 6 hours, filter, and add sufficient water to produce 250 ml.
Storage. Store at a temperature between 2 and 8 °C, protected from light.

Phthalate buffer, pH 3.4, TS
Procedure. Dissolve 2.04 g of potassium hydrogen phthalate R in 50 ml of carbon-dioxide-free water R, add 10.40 ml of hydrochloric acid (0.2 mol/l) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 ml.

Phthalate buffer, pH 3.5, TS

Procedure. Dissolve 2.04 g of potassium hydrogen phthalate R in 50 ml of carbon-dioxide-free water R, add 8.40 ml of hydrochloric acid (0.2 mol/l) VS, and dilute with sufficient carbon-dioxide-free water R to produce 200 ml.

Phthalate buffer, pH 4.0, TS

Procedure. Dissolve 2.042 g of potassium hydrogen phthalate R in 50 ml of water, add 0.40 ml of sodium hydroxide (0.2 mol/l) VS, and dilute to 200 ml with water.

Phthalic anhydride R. $C_8H_4O_3$.

Description. White lustrous needles.

Solubility. Slightly soluble in water, more soluble in hot water; soluble in ethanol (~750 g/l) TS and ether R.

Melting temperature. About 130 °C.

Phthalic anhydride/pyridine TS

Procedure. Add 42 g of phthalic anhydride R, accurately weighed, to 300 ml of freshly distilled pyridine R (refluxed with barium oxide R) containing less than 1 mg/ml of water in a glass-stoppered 1000 ml flask. Use a dark flask or otherwise prevent exposure to light. Shake vigorously until complete solution is effected, and allow to stand overnight for completion of the reaction.

Note: Phthalic anhydride/pyridine TS must be freshly prepared.

Piperazine adipate RS. International Chemical Reference Substance.

Piperazine citrate RS. International Chemical Reference Substance.

Piperazine hydrate R. Piperazine hexahydrate; $C_4H_{10}N_2,6H_2O$.

Description. Colourless, glossy, deliquescent crystals.

Melting point. 44 °C.

Piperidine R. $C_5H_{11}N$.

Description. A colourless to yellowish liquid; odour, characteristic.

Miscibility. Miscible with water and ethanol (~750 g/l) TS.

Mass density. ρ_{20} = about 0.86 kg/l.

Refractive index. n_D^{20} = about 1.454.

Boiling temperature. About 106 °C.

Congealing temperature. Between 12 and 15 °C.

Plasma substrate R

Note: Use water-repellent equipment (made from materials such as suitable plastics or suitably silicone-treated glass) for taking and handling blood.

Procedure. Collect a sufficient volume of blood from each of at least 5 sheep. A 285-ml volume of blood collected into 15 ml of anticoagulant solution is recommended but smaller volumes may be collected. The blood should be

taken either from a live animal or at the time of slaughter, using a needle attached to a cannula that is long enough to reach the bottom of the collecting flask. Discard the first few ml and collect only free-flowing blood. Collect the blood in a sufficient quantity of an anticoagulant solution containing 8.7 g of sodium citrate R and 4 mg of aprotinin R in 100 ml of water to give a final ratio of blood to anticoagulant solution of 19 to 1. During and immediately after collection, swirl the flask gently to ensure mixing but do not allow frothing to occur. When collection is complete, close the flasks and cool to a temperature between 10 and 15 °C. Then pool the contents of all the flasks, with the exception of any that shows obvious haemolysis or clots, and keep the pooled blood at 10–15 °C. Within 4 hours of collection, centrifuge the pooled and cooled blood at a speed of 1000–2000 *g*[1] for 30 minutes. Separate the supernatant liquid and centrifuge again at 5000 *g* for 30 minutes (*Note*: Faster centrifugation (20 000 *g*) may be necessary to clarify the plasma, but filtration procedures should not be used.) Separate the supernatant liquid and immediately mix thoroughly and distribute the plasma substrate R into small stoppered containers in portions sufficient for a complete heparin assay (10–30 ml). Without delay, rapidly cool to a temperature below –70 °C by immersing the containers in liquid nitrogen and store at a temperature below –30 °C. The plasma is suitable for use as plasma substrate R in the assay for heparin if, under the conditions of the assay, it gives a clotting time appropriate to the method of detection used, and if it provides reproducible, steep, log dose – response curves. Just before use thaw the quantity of plasma substrate R required in a water-bath at 37 °C and gently swirl until thawing is complete; once thawed it should be kept between 10 and 20 °C and used without delay. The thawed plasma substrate R may be slightly centrifuged if necessary, but do not use any filtration procedures.

Platinic chloride (60 g/l) TS. A solution of platinic chloride R containing about 63 g of H_2PtCl_6, per litre.

Platinic chloride R. $H_2PtCl_6,6H_2O$ (SRIP, 1963, p. 144).

Polydimethylsiloxane R. A commercially available reagent of suitable grade for use in gas chromatography.

Polysorbate 80 R. The mono ester of oleic acid and tripolyethyleneglycol 300-sorbitan ether.

Description. Lemon to amber coloured, oily liquid.

Miscibility. Miscible with water, producing an odourless and nearly colourless solution. Miscible with ethanol (~750 g/l) TS, ethyl acetate R, and vegetable oils; immiscible with mineral oils.

[1] Acceleration due to gravity = 9.81 m/s^2.

Potassio-cupric tartrate TS

Procedure. Dissolve 7 g of copper(II) sulfate R in sufficient water to produce 100 ml. Separately dissolve 35 g of potassium sodium tartrate R and 10 g of sodium hydroxide R in 100 ml of water. Shortly before use, mix together equal volumes of both solutions.

Potassio-mercuric iodide TS

Procedure. Dissolve 1.355 g of mercuric chloride R in 60 ml of water; separately dissolve 5 g of potassium iodide R in 20 ml of water; mix the two solutions and dilute to 100 ml with water.

Potassio-mercuric iodide, alkaline, TS

Procedure. Dissolve 3.5 g of potassium iodide R and 1.25 g of mercuric chloride R in 80 ml of water, add while stirring a cold saturated solution of mercuric chloride R in water until a slight red precipitate remains. Then add 12 g of sodium hydroxide R and mix to dissolve, add a little more of the saturated solution of mercuric chloride R and sufficient water to produce 100 ml; allow to stand for 24 hours and decant the clear liquid.

Potassium acetate R. $C_2H_3KO_2$ (SRIP, 1963, p. 144).

Potassium acetate TS

Procedure. Dissolve 100 g of potassium acetate R in sufficient glacial acetic acid R to produce 1000 ml.

Potassium antimonate R. $KSbO_3$ (SRIP, 1963, p. 145).

Potassium antimonate TS

Procedure. Boil 2 g of potassium antimonate R with 95 ml of water until it has dissolved. Cool rapidly and add 50 ml of potassium hydroxide (1 mol/l) VS and 5 ml of sodium hydroxide (1 mol/l) VS. Allow to stand for 24 hours and dilute with sufficient water to produce 150 ml.

Sensitivity to sodium. To 10 ml add 7 ml of sodium hydroxide (0.1 mol/l) VS; a white, crystalline precipitate is formed within 15 minutes.

Note. Potassium antimonate TS must be freshly prepared.

Potassium bicarbonate R. $KHCO_3$ (SRIP, 1963, p. 145).

Potassium bromate (0.00833 mol/l) VS. Potassium bromate R, dissolved in water to contain 1.392 g of $KBrO_3$ in 1000 ml.

Potassium bromate (0.0167 mol/l) VS. Potassium bromate R, dissolved in water to contain 2.784 g of $KBrO_3$ in 1000 ml.

Potassium bromate (0.0333 mol/l) VS. Potassium bromate R, dissolved in water to contain 5.562 g of $KBrO_3$ in 1000 ml.

Potassium bromate (50 g/l) TS. A solution of potassium bromate R containing about 50 g of $KBrO_3$ per litre.

Potassium bromate R. $KBrO_3$ (SRIP, 1963, p. 147).

Potassium bromide (0.119 g/l) TS. A solution of potassium bromide R containing about 0.1190 g of KBr per litre.

Potassium bromide (100 g/l) TS. A solution of potassium bromide R containing about 100 g of KBr per litre.

Potassium bromide (125 g/l) TS. A solution of potassium bromide R containing about 125 g of KBr per litre.

Potassium bromide IR. Potassium bromide R that complies with the following test: The infrared absorption spectrum of a disc prepared as described in Method 3 under 1.7 Spectrophotometry in the infrared region, from potassium bromide R, previously dried at 250 °C for 1 hour, has a substantially flat baseline over the range 4000–670 cm^{-1}; it exhibits no maxima with an absorbance greater than 0.1 above the baseline, with the exception of maxima due to water at 3440 and 1630 cm^{-1}.

Potassium bromide R. KBr (SRIP, 1963, p. 148).

Potassium carbonate R. $K_2CO_3,1\frac{1}{2}H_2O$.
Description. Small granular crystals.
Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Potassium carbonate, anhydrous, R. K_2CO_3.
Description. Granules or a granular powder; hygroscopic.
Solubility. Soluble in 1 part of water; practically insoluble in ethanol (~750 g/l) TS.

Potassium chloride (100 g/l) TS. A solution of potassium chloride R containing about 100 g of KCl per litre.

Potassium chloride (350 g/l) TS. A saturated solution of potassium chloride R containing about 350 g/l of KCl.

Potassium chloride IR. Potassium chloride R that complies with the following test: The infrared absorption spectrum of a disc prepared as described in Method 3 under 1.7 Spectrophotometry in the infrared region, from potassium chloride R, previously dried at 250 °C for 1 hour, has a substantially flat baseline over the range 4000—670 cm^{-1}; it exhibits no maxima with an absorbance greater than 0.1 above the baseline, with the exception of maxima due to water at 3440 and 1630 cm^{-1}.

Potassium chloride R. KCl (SRIP, 1963, p. 151).

Potassium chromate (100 g/l) TS. A solution of potassium chromate R containing about 97 g of K_2CrO_4 per litre (approximately 0.5 mol/l).

Potassium chromate R. K_2CrO_4 (SRIP, 1963, p. 152).

Potassium cyanide (100 g/l) TS. A solution of potassium cyanide R containing about 100 g of KCN per litre.

Potassium cyanide (50 g/l) TS. A solution of potassium cyanide R containing about 50 g of KCN per litre.

Potassium cyanide PbTS

Procedure. Dissolve 10 g of potassium cyanide R in 90 ml of water, add 2 ml of hydrogen peroxide (~60 g/l) TS, allow to stand for 24 hours, dilute with water to 100 ml, and filter.

Potassium cyanide R. KCN (SRIP, 1963, p. 153).

Potassium dichromate (0.0167 mol/l) VS. Potassium dichromate R, dissolved in water to contain 4.904 g of $K_2Cr_2O_7$ in 1000 ml.

Potassium dichromate (100 g/l) TS. A solution of potassium dichromate R containing about 98 g of $K_2Cr_2O_7$ per litre (approximately 0.4 mol/l).

Potassium dichromate R. $K_2Cr_2O_7$ (SRIP, 1963, p. 154).

Potassium dichromate R1. Potassium dichromate R containing not less than 99.9% of $K_2Cr_2O_7$.

Potassium dichromate TS

Procedure. Dissolve about 60 mg, accurately weighed and previously dried at 130 °C, of potassium dichromate R1 in sufficient sulfuric acid (0.005 mol/l) VS to produce 1000.0 ml.

Potassium dichromate TS2

Procedure. Dissolve 1 g of potassium dichromate R in 60 ml of water and cautiously add 7.5 ml of sulfuric acid (~1760 g/l) TS.

Potassium dichromate TS3

Procedure. Dissolve 0.5 g of potassium dichromate R in sufficient sulfuric acid (~100 g/l) TS to produce 100 ml.

Potassium dihydrogen phosphate (100 g/l) TS. A solution of potassium dihydrogen phosphate R containing about 100 g of KH_2PO_4 per litre.

Potassium dihydrogen phosphate (13.6 g/l) TS. A solution of potassium dihydrogen phosphate R containing 13.6 g of KH_2PO_4 per litre (0.1 mol/l).

Potassium dihydrogen phosphate (27.2 g/l) TS. A solution of potassium dihydrogen phosphate R containing 27.2 g of KH_2PO_4 per litre (0.2 mol/l).

Potassium dihydrogen phosphate (70 g/l) TS. A solution of potassium dihydrogen phosphate R containing about 70 g of KH_2PO_4 per litre.

Potassium dihydrogen phosphate R. KH_2PO_4 (SPRI, 1963, p. 155).

Potassium ferricyanide (10 g/l) TS

Procedure. Wash about 1 g of crystalline potassium ferricyanide R with a little water and dissolve the washed crystals in sufficient water to produce 100 ml.

Note: Potassium ferricyanide (10 g/l) TS must be freshly prepared.

Potassium ferricyanide (50 g/l) TS

Procedure. Wash about 5 g of crystalline potassium ferricyanide R with a little water and dissolve the washed crystals in sufficient water to produce 100 ml.

Note: Potassium ferricyanide (50 g/l) TS must be freshly prepared.

Potassium ferricyanide R. $K_3Fe(CN)_6$ (SRIP, 1963, p. 156).

Potassium ferrocyanide (45 g/l) TS. A solution of potassium ferrocyanide R containing about 50 g of $K_4Fe(CN)_6$ per litre.

Potassium ferrocyanide R. $K_4Fe(CN)_6,3H_2O$ (SRIP, 1963, p. 156).

Potassium hydrogen phthalate R. $C_8H_5KO_4$ (SRIP, 1963, p. 157).

Potassium hydrogen phthalate standard TS

Procedure. Dissolve 10.21 g of potassium hydrogen phthalate R, previously dried at 120 °C, in sufficient carbon-dioxide-free water R to produce 1000 ml. The pH of this solution is defined as having the value of 4.000 at 15 °C.

Potassium hydrogen sulfate R. $KHSO_4$ (SRIP, 1963, p. 146).

Potassium hydrogen tartrate R. $C_4H_5KO_6$ (SRIP, 1963, p. 158).

Potassium hydrogen tartrate standard TS

Procedure. Add 2 g of potassium hydrogen tartrate R to 100 ml of carbon-dioxide-free water R contained in a glass-stoppered flask and shake the flask

vigorously. Let the temperature of the solution reach room temperature, allow the solid to settle, and remove it by filtration or decantation.

Note: Potassium hydrogen tartrate standard TS must be freshly prepared.

Potassium hydroxide (~110 g/l) TS. A solution of potassium hydroxide R containing about 112 g/l of KOH (approximately 2 mol/l).

Potassium hydroxide (~400 g/l) TS. A solution of potassium hydroxide R containing about 400 g of KOH per litre.

Potassium hydroxide (~560 g/l) TS. A solution of potassium hydroxide R containing about 560 g of KOH per litre.

Potassium hydroxide (0.01 mol/l) VS. Potassium hydroxide R, dissolved in water to contain 0.5610 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/l) VS.

Potassium hydroxide (0.1 mol/l) VS. Potassium hydroxide R, dissolved in water to contain 5.610 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/l) VS.

Potassium hydroxide (0.5 mol/l) VS. Potassium hydroxide R, dissolved in water to contain 28.05 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/l) VS.

Potassium hydroxide (1 mol/l) VS. Potassium hydroxide R, dissolved in water to contain 56.10 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: Dry about 5 g of potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals, they should be crushed before drying. Dissolve in 75 ml of carbon-dioxide-free water R and titrate with the potassium hydroxide solution, using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 ml of potassium hydroxide (1 mol/l) VS. Standard solutions of potassium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should, therefore, be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Potassium hydroxide R. KOH (SRIP, 1963, p. 159).

Potassium hydroxide/ethanol (0.02 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (~710 g/l) TS to contain 1.122 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/l) VS.

Potassium hydroxide/ethanol (0.1 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (~710 g/l) TS to contain 5.610 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/l) VS.

Potassium hydroxide/ethanol (0.5 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (~710 g/l) TS to contain 28.05 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.5 mol/l solution in the following manner: Dilute 25.0 ml of hydrochloric acid (0.5 mol/l) VS with 50 ml of water and titrate with the potassium hydroxide/ethanol solution, using phenolphthalein/ethanol TS as indicator.

Potassium hydroxide/ethanol (1 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (~710 g/l) TS to contain 56.10 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/l) VS.

Potassium hydroxide/ethanol TS1

Procedure. Dissolve 40 g of potassium hydroxide R in 20 ml of water and add sufficient ethanol (~750 g/l) TS to produce 1000 ml. Allow to stand overnight, and pour off the clear liquid.

Potassium hydroxide/ethanol TS2

Procedure. Dissolve 112 g of potassium hydroxide R in sufficient ethanol (~710 g/l) TS to produce 1000 ml (approximately 2 mol/l).

Potassium hydroxide/methanol TS

Procedure. Dissolve 30 g of potassium hydroxide R in sufficient methanol R to produce 1000 ml.

Potassium iodate (0.01 mol/l) VS. Potassium iodate R, dissolved in water to contain 2.140 g of KIO_3 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium iodate (0.05 mol/l) VS.

Potassium iodate (0.05 mol/l) VS. Potassium iodate R, dissolved in water to contain 10.70 g of KIO_3 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/l solution in the following manner. Place 10.0 ml of the potassium iodate solu-

tion in a glass-stoppered flask, dilute with 200 ml of water, add 2 g of potassium iodide R, and 25 ml of sulfuric acid (~100 g/l) TS. Allow the solution to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, adding 3 ml of starch TS as the end-point is approached. Correct for a blank determined on the same quantities of the same reagents.

Potassium iodate (3.6 mg/l) TS. A freshly prepared solution of potassium iodate R containing 3.6 mg of KIO_3 per litre.

Potassium iodate R. KIO_3 (SRIP, 1963, p. 160).

Potassium iodide (100 g/l) TS. A solution of potassium iodide R containing about 100 g of KI per litre.

Potassium iodide (160 g/l) TS. A solution of potassium iodide R containing about 160 g of KI per litre.

Potassium iodide (300 g/l) TS. A solution of potassium iodide R containing about 300 g of KI per litre.

Potassium iodide (400 g/l) TS. A solution of potassium iodide R containing about 400 g of KI per litre.

Potassium iodide (60 g/l) TS. A solution of potassium iodide R containing about 60 g of KI per litre.

Potassium iodide (80 g/l) TS. A solution of potassium iodide R containing about 83 g/l of KI (approximately 0.5 mol/l).

Potassium iodide AsR. Potassium iodide R that complies with the following test: Dissolve 10 g of potassium iodide R in 25 ml of hydrochloric acid (~250 g/l) AsTS and 35 ml of water, add 2 drops of stannous chloride AsTS and apply the general test for arsenic; no visible stain is produced.

Potassium iodide R. KI (SRIP, 1963, p. 161).

Potassium iodide/starch TS1

Procedure. Dissolve 10 g of potassium iodide R in 95 ml of water and add to it 5 ml of starch TS.

Note: Potassium iodide/starch TS1 must be freshly prepared.

Potassium iodobismuthate TS1

Procedure. Dissolve 100 g of tartaric acid R in 400 ml of water and add 8.5 g of bismuth oxynitrate R. Shake the solution for 1 hour, add 200 ml of potassium iodide (400 g/l) TS, and mix. Allow to stand for 24 hours and filter.

Potassium iodobismuthate TS2
Procedure. Dissolve 100 g of tartaric acid R in 500 ml of water and add 50 ml of potassium iodobismuthate TS1.

Potassium iodobismuthate/acetic acid TS
Procedure. Dissolve 8 g of potassium iodide R in 20 ml of water and add to it a solution composed of 0.85 g of bismuth oxynitrate R dissolved in 40 ml of water and 10 ml of glacial acetic acid R.

Potassium iodoplatinate TS
Procedure. Dissolve 2.5 g of platinic chloride R in 50 ml of water, add 45 ml of a 0.1 g/ml solution of potassium iodide R, and dilute to 100 ml with water.
Storage. Store in amber glass containers.

Potassium iodoplatinate TS2
Procedure. Dissolve 0.25 g of platinic chloride R in 2.5 ml of water, add 45 ml of potassium iodide (100 g/l) TS, and dilute with sufficient acetone R to produce 100 ml.

Potassium nitrate R. KNO_3 (SRIP, 1963, p. 162).

Potassium nitrite (100 g/l) TS. A solution of potassium nitrite R containing about 100 g/l of KNO_2.

Potassium nitrite R. KNO_2.
Description. White or slightly yellow, deliquescent granules or rods.
Solubility. Soluble in 0.35 part of water; slightly soluble in ethanol (~750 g/l) TS.

Potassium periodate R. KIO_4 (SRIP, 1963, p. 164).

Potassium periodate TS
Procedure. To 2.8 g of potassium periodate R add 200 ml of water followed by 20 ml of sulfuric acid (~1760 g/l) TS added drop by drop while shaking to effect solution; cool and add sufficient water to produce 1000 ml.

Potassium permanganate (0.0002 mol/l) VS. Potassium permanganate R, dissolved in water to contain 31.61 mg of $KMnO_4$ in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under "Potassium permanganate (0.02 mol/l) VS".

Potassium permanganate (0.002 mol/l) VS. Potassium permanganate R, dissolved in water to contain 0.3161 g of $KMnO_4$ in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium permanganate (0.02 mol/l) VS.

Potassium permanganate (0.02 mol/l) VS. Potassium permanganate R, dissolved in water to contain 3.161 g of $KMnO_4$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.02 mol/l solution in the following manner: Dissolve about 0.2 g, accurately weighed, of sodium oxalate R, previously dried to constant weight at 110 °C, in 250 ml of water. Add 7 ml of sulfuric acid (~1760 g/l) TS, heat to about 70 °C and then slowly add the permanganate solution from a burette, with constant stirring, until a pale pink colour, which persists for 15 seconds, is produced. The temperature at the conclusion of the titration should be not less than 60 °C. Every 6.7 mg of sodium oxalate are equivalent to 1 ml of potassium permanganate (0.02 mol/l) VS. Potassium permanganate solutions should be restandardized frequently.

Storage. Store the solution in tightly closed containers, protected from light.

Potassium permanganate (~25 g/l) TS. A solution of potassium permanganate R containing about 25 g of $KMnO_4$ per litre.

Potassium permanganate (~10 g/l) TS. A solution of potassium permanganate R containing about 10 g/l of $KMnO_4$.

Potassium permanganate (~1 g/l) TS. A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre.

Potassium permanganate R. $KMnO_4$ (SRIP, 1963, p. 165).

Potassium permanganate, basic (~5 g/l) TS

A solution of potassium permanganate R containing about 5 g of $KMnO_4$ per litre of sodium hydroxide (~40 g/l) TS.

Potassium permanganate, basic (~1 g/l) TS

A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre of sodium hydroxide (~40 g/l) TS.

Potassium permanganate/phosphoric acid TS

Procedure. Dissolve 3 g of potassium permanganate R in a mixture of 15 ml of phosphoric acid (~1440 g/l) TS and 70 ml of water, and dilute to 100 ml with water.

Potassium sodium tartrate R [sodium potassium tartrate R]. $C_4H_4KNaO_6,4H_2O$ (SRIP, 1963, p. 193).

Potassium sulfate (0.1 g/l) TS. A solution of potassium sulfate R containing about 0.1 g of K_2SO_4 per litre.

Potassium sulfate (174 mg/l) TS

Procedure. Dissolve 174 mg, accurately weighed, of potassium sulfate R in sufficient water to produce 1000 ml.

Potassium sulfate R. K_2SO_4 (SRIP, 1963, p. 165).

Potassium tetraoxalate R. $C_4H_3KO_8,2H_2O$ (SRIP, 1963, p. 166).

Potassium tetraoxalate standard TS

Procedure. Dissolve 25.42 g of potassium tetraoxalate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Potassium thiocyanate (200 g/l) TS. A solution of potassium thiocyanate R containing 200 g of KCNS per litre.

Potassium thiocyanate R. KCNS. Contains not less than 99.0% of KCNS, calculated with reference to the dried substance.

Description. Colourless crystals.

Solubility. Soluble in 0.5 part of water and in 15 parts of dehydrated ethanol R.

Alkalinity. A 0.1 g/ml solution in carbon-dioxide-free water R is not alkaline to bromothymol blue/ethanol TS.

Ammonia. Boil 1.0 g with 5 ml of sodium hydroxide (~80 g/l) TS; no ammonia is evolved.

Chlorides. Dissolve 1.0 g in a solution of 1 g of ammonium nitrate R in 30 ml of hydrogen peroxide (~60 g/l) TS containing not more than 1 µg/g of chlorides, add 1 g of sodium hydroxide R, and warm gently; when the vigorous reaction subsides add a further 30 ml of hydrogen peroxide (~60 g/l) TS and boil for 2 minutes. Cool, add 5 ml of nitric acid (~1000 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; any opalescence produced is not greater than that produced by treating 1 ml of hydrochloric acid (0.01 mol/l) VS in the same manner.

Sulfates. Dissolve 0.50 g in 20 ml of water and proceed as described under 2.2.2 Limit test for sulfates; not more than 1.0 mg/g.

Other sulfur compounds. Dissolve 1.0 g in 50 ml of water, add 2 ml of hydrochloric acid (~70 g/l) TS, and titrate with iodine (0.05 mol/l) VS; not more than 0.5 ml of iodine (0.05 mol/l) VS is required.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 20 mg/g.

Assay. Dissolve about 0.4 g, accurately weighed, in 50 ml of water, add 5 ml of nitric acid (~1000 g/l) TS, 50 ml of silver nitrate (0.1 mol/l) VS, and 5 ml of ferric ammonium sulfate (45 g/l) TS, and titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 9.718 mg of KCNS.

Note: Potassium thiocyanate R is deliquescent.

Praziquantel RS. International Chemical Reference Substance.

Prednisolone acetate RS. International Chemical Reference Substance.

Prednisolone RS. International Chemical Reference Substance.

Prednisolone sodium phosphate RS. International Chemical Reference Substance.

Prednisolone succinate RS. International Chemical Reference Substance.

Primaquine diphosphate RS. International Chemical Reference Substance.

Probenecid RS. International Chemical Reference Substance.

Procaine hydrochloride RS. International Chemical Reference Substance.

Procarbazine hydrochloride RS. International Chemical Reference Substance.

Progesterone RS. International Chemical Reference Substance.

Proguanil hydrochloride RS. International Chemical Reference Substance.

2-Propanol R [*iso*-propanol R]; isopropyl alcohol; C_3H_8O (SRIP, 1963, p. 167).

1-Propanol R. *n*-Propanol; propan-1-ol, C_3H_8O.
Description. A clear, colourless liquid.
Miscibility. Miscible with water and ethanol (~750 g/l) TS.
Boiling range. Not less than 95% distils between 95 and 98 °C.
Mass density. ρ_{20} = about 0.803 kg/l.
Residue on evaporation. Evaporate on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.1 mg/g.

Propranolol hydrochloride RS. International Chemical Reference Substance.

Propylene glycol R. $C_3H_8O_2$ (SRIP, 1963, p. 168).

Protionamide RS. International Chemical Reference Substance.

Pyrantel embonate RS. International Chemical Reference Substance.

Pyrazinamide RS. International Chemical Reference Substance.

Pyridine R. C_5H_5N (SRIP, 1963, p. 169).

Pyridine, anhydrous, R. Pyridine R that has been dried by allowing it to stand over sodium hydroxide R.

Pyridine/acetic anhydride TS
Procedure. Mix 3 volumes of freshly distilled pyridine R with 1 volume of freshly distilled acetic anhydride R.
Note: Pyridine/acetic anhydride TS must be freshly prepared.

Pyridostigmine bromide RS. International Chemical Reference Substance.

Pyrimethamine RS. International Chemical Reference Substance.

Pyrogallol R. Pyrogallic acid; 1,2,3-trihydroxybenzene, $C_6H_6O_3$ (SRIP, 1963, p. 170).

Pyrogallol, alkaline, TS
Procedure. Dissolve 0.5 g of pyrogallol R in 2 ml of water. Dissolve separately 12 g of potassium hydroxide R in 8 ml of water. Immediately before use mix the two solutions.

Quinaldine red R. 2-(*p*-Dimethylaminostyryl)quinoline ethiodide; $C_{21}H_{23}IN_2$.
Description. A dark blue-black powder.
Solubility. Sparingly soluble in water; freely soluble in ethanol (~750 g/l) TS.
Melting temperature. About 260 °C with decomposition.

Quinaldine red/ethanol TS
Procedure. Dissolve 0.1 g of quinaldine red R in 100 ml of ethanol (~750 g/l) TS.

Quinaldine red/methanol TS
Procedure. Dissolve 1.0 g of quinaldine red R in sufficient methanol R to produce 100 ml.

Quinhydrone R. *p*-Benzoquinone compound with hydroquinone (1:1); $C_{12}H_{10}O_4$.
Description. Dark green, lustrous crystals or a crystalline powder.
Melting point. About 171 °C.

Quinhydrone/methanol TS
Procedure. Dissolve 2.5 g of quinhydrone R in sufficient methanol R to produce 100 ml.

Quinine R. $C_{20}H_{24}N_2O_2$.
Description. A white, microcrystalline powder; odourless.
Solubility. Very slightly soluble in water; slightly soluble in boiling water; very soluble in ethanol (~750 g/l) TS; soluble in ether R and benzene R.
Melting temperature. About 175 °C.

Identification. Very dilute solutions containing sulfuric acid (~100 g/l) TS show a blue fluorescence. Acid solutions are levorotatory. Dissolve about 5 mg in a mixture of 5 ml of water and 0.3 ml of hydrochloric acid (~70 g/l) TS. Mix the solution with 0.2 ml of bromine TS1 and add 1 ml of ammonia (~35 g/l) TS; an emerald green colour is produced.

Red stock standard TS

Procedure. To 40.5 ml of cobalt colour TS, add 6.1 ml of copper colour TS, 6.3 ml of dichromate colour TS, 12.0 ml of iron colour TS, dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Resazurin sodium (1 g/l) TS. A solution of resazurin sodium R containing about 1 g/l of $C_{12}H_6NNaO_4$.

Note: Resazurin sodium (1 g/l) TS must be freshly prepared.

Resazurin sodium R. $C_{12}H_6NNaO_4$ (SRIP, 1963, p. 170).

Reserpine RS. International Chemical Reference Substance.

Resorcinol (20 g/l) TS. A solution of resorcinol R containing 20 g/l of $C_6H_6O_2$.

Resorcinol R. 1,3-Dihydroxybenzene, $C_6H_6O_2$ (SRIP, 1963, p. 171).

Resorcinol/toluene TS

Procedure. Shake 0.2 g of resorcinol R with 100 ml of toluene R until saturated, and decant.

Note: Resorcinol/toluene TS should be prepared immediately before use.

Retinol acetate RS. International Chemical Reference Substance.

Retinol palmitate RS. International Chemical Reference Substance.

Retinol propionate RS. International Chemical Reference Substance.

Riboflavin RS. International Chemical Reference Substance.

Rifampicin quinone RS. International Chemical Reference Substance.

Rifampicin RS. International Chemical Reference Substance.

Ritonavir RS. International Chemical Reference Substance.

Salbutamol RS. International Chemical Reference Substance.

Salbutamol sulfate RS. International Chemical Reference Substance.

Salicylaldehyde R. $C_7H_6O_2$.
Description. A clear, colourless, oily liquid; odour, bitter, almond-like.
Solubility. Slightly soluble in water; soluble in ethanol (~750 g/l) TS and ether R.
Relative density. $d_4^{20} = 1.17$.

Salicylaldehyde TS
Procedure. Mix 2 g of salicylaldehyde R with 100 ml of methanol R and add 0.1 ml of hydrocloric acid (~420 g/l) TS.

Salicylic acid R. $C_7H_6O_3$. Salicylic acid as described in the monograph for "Salicylic acid".

Saline TS. A sterile solution of sodium chloride R containing about 9 g/l of NaCl. Sterilization by heating in a steam autoclave at 120 °C for 30 minutes is suitable.

Saquinavir mesilate RS. International Chemical Reference Substance.

Saquinavir RS. International Chemical Reference Substance.

Selenious acid R. H_2SeO_3. Contains not less than 93% of H_2SeO_3.
Description. Colourless or white crystals.
Solubility. Soluble in water and ethanol (~750 g/l) TS.
Assay. Transfer about 0.1 g, accurately weighed, to a glass-stoppered flask, and dissolve in 50 ml of water. Add 10 ml of potassium iodide (300 g/l) TS and 5 ml of hydrochloric acid (~420 g/l) TS, mix, insert the stopper into the flask, and allow to stand for 10 minutes. Dilute with 50 ml of water, add 3 ml of starch TS, and titrate with sodium thiosulfate (0.1 mol/l) VS until the colour is no longer diminished, then titrate with iodine (0.05 mol/l) VS to a blue colour. Subtract the volume of iodine (0.05 mol/l) VS from the volume of sodium thiosulfate (0.1 mol/l) VS equivalent to selenious acid. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 3.225 mg of H_2SeO_3.
Note: Selenious acid R effloresces in dry air and is hygroscopic in moist air.

Selenious acid/sulfuric acid TS
Procedure. Dissolve 10 mg of selenious acid R in 2 ml of sulfuric acid (~1760 g/l) TS.

Selenium R. Se (SRIP, 1963, p. 172).
Caution. Selenium vapours are toxic.

Silica gel for chromatography R. A very finely divided (3–10µm) silica gel. The particle size is indicated after the name of the reagent in the tests where it is used.

Description. A fine, white, homogeneous powder.
Solubility. Practically insoluble in water and ethanol (~750 g/l) TS.

Silica gel R1. Silica gel G.
Description. A white, homogeneous powder.
Composition. A mixture of silica gel (particle size 10–40 µm) and calcium sulfate, hemihydrate (about 130 g/kg).

Silica gel R2. Silica gel HF(UV254).
Description. A white, homogeneous powder.
Composition. Silica gel (particle size 10–40 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel R3. Silica gel H.
Description. A white, homogeneous powder.
Particle size. 10–40 µm.

Silica gel R4. Silica gel GF (UV 254).
Description. A white, homogeneous powder.
Composition. A mixture of silica gel (particle size 10–40 µm) and calcium sulfate, hemihydrate (about 130 g/kg) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel R5. Silica gel 60.
Description. A white, homogeneous powder.
Average pore size. 6 nm.

Silica gel R6. Silica gel 60 (UV 254).
Description. A white, homogeneous powder.
Average pore size. 6 nm.
Composition. Silica gel (average particle size 15 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg).

Silica gel for chromatography, hexadecylamidylsilyl
Particles of silica gel, the surface of which has been modified with chemically bonded hexadecylamidylsilyl groups.

Silica gel for chromatography, octadecylsilyl, base deactivated
A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Silica gel, desiccant, R
Description. An amorphous, partly hydrated SiO_2, occurring in glassy granules of varying sizes. It is frequently coated with a substance that changes colour when the capacity to absorb water is exhausted. Such coloured products

may be regenerated (i.e. may regain their capacity to absorb water) by heating at 110 °C until the gel assumes the original colour.

Loss on ignition. Ignite 2 g, accurately weighed, at 950 ± 50 °C to constant weight; the loss is not more than 60 mg/g.

Water absorption. Place about 10 g in a tared weighing-bottle, and weigh. Then place the bottle, with the cover removed, for 24 hours in a closed container in which the atmosphere is maintained at 80% relative humidity by being in equilibrium with sulfuric acid having a relative density of 1.19. Weigh again; the increase in weight is not less than 310 mg/g.

Silver nitrate (0.001 mol/l) VS. Silver nitrate R, dissolved in water to contain 0.1699 g of $AgNO_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Silver nitrate (0.1 mol/l) VS".

Silver nitrate (0.01 mol/l) VS. Silver nitrate R, dissolved in water to contain 1.699 g of $AgNO_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under silver nitrate (0.1 mol/l) VS.

Silver nitrate (0.05 mol/l) VS. Silver nitrate R, dissolved in water to contain 8.494 g of $AgNO_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under "Silver nitrate (0.1 mol/l) VS".

Silver nitrate (0.1 mol/l) VS. Silver nitrate R, dissolved in water to contain 16.99 g of $AgNO_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Dilute 40.0 ml of the silver nitrate solution with 100 ml of water. Heat the solution and add slowly, with continuous stirring, hydrochloric acid (~70 g/l) TS until precipitation of the silver is complete. Boil the mixture cautiously for about 5 minutes, then allow it to stand in the dark until the precipitate has settled and the supernatant liquid has become clear. Transfer the precipitate completely to a tared filtering crucible and wash it with small portions of water that has been slightly acidified with nitric acid (~1000 g/l) TS. Dry the precipitate to constant weight at 110 °C. From the weight of silver chloride calculate the concentration of the silver nitrate solution in mol/l. Protect the silver chloride from light as much as possible during the determination.

Silver nitrate (100 g/l) TS. A solution of silver nitrate R containing 100 g of $AgNO_3$ per litre.

Silver nitrate (40 g/l) TS. A solution of silver nitrate R containing about 42.5 g/l of $AgNO_3$ (approximately 0.25 mol/l).

Silver nitrate R. $AgNO_3$ (SRIP, 1963, p. 173).

Silver nitrate/methanol TS

Procedure. Prepare a saturated solution of silver nitrate R in methanol R.

Silver oxide R. Ag_2O.

Description. A brownish-black, heavy powder; odourless.

Solubility. Practically insoluble in water; freely soluble in nitric acid (~130 g/l) TS and ammonia (~260 g/l) TS.

Substances insoluble in nitric acid. Dissolve 5 g in a mixture of 5 ml of nitric acid (~1000 g/l) TS and 10 ml of water, dilute to about 65 ml with water, and filter any undissolved residue on a tared filtering crucible (retain the filtrate for the test for substances not precipitated by hydrochloric acid). Wash the crucible with water until the last washing shows no opalescence with 1 drop of hydrochloric acid (~250 g/l) TS, and dry to constant weight at 105 °C; not more than 0.2 mg/g.

Substances not precipitated by hydrochloric acid. Dilute the filtrate obtained in the test for substances insoluble in nitric acid to 250 ml with water, heat to boiling, and add dropwise sufficient hydrochloric acid (~250 g/l) TS to precipitate all of the silver (about 5 ml), avoiding any great excess. Cool, dilute to 300 ml with water, and allow to stand overnight. Filter, evaporate 200 ml of the filtrate to dryness in a suitable tared porcelain dish, and ignite; not more than 0.5 mg/g.

Alkalinity. Heat 2 g with 40 ml of water on a water-bath for 15 minutes, cool, and dilute to 50 ml with water. Filter, discarding the first 10 ml of the filtrate. To 25 ml of the subsequent filtrate add 2 drops of phenolphthalein/ethanol TS, and titrate with hydrochloric acid (0.02 mol/l) VS to the disappearance of any pink colour; not more than 0.20 ml is required.

Silver standard (5 µg Ag/ml) TS

Procedure. Dissolve 39.5 mg of silver nitrate R in sufficient water to produce 100 ml. Dilute 1.0 ml of this solution to 100 ml with water.

Soda lime R. (SRIP, 1963, p. 174).

Sodium 1,2-naphthoquinone-4-sulfonate (5 g/l) TS. A solution of sodium 1,2-naphthoquinone-4-sulfonate R containing about 5 g of $C_{10}H_5NaO_5S$ per litre.

Sodium 1,2-naphthoquinone-4-sulfonate R. $C_{10}H_5NaO_5S$.

Description. A yellow or orange, crystalline powder.

Solubility. Soluble in water; insoluble in ethanol (~750 g/l) TS.

Sodium acetate (0.04 mol/l) VS. Sodium acetate R, dissolved in water to contain 3.281 g of $C_2H_3NaO_2$ in 1000 ml.

Sodium acetate (150 g/l) TS. A solution of sodium acetate R containing about 150 g/l of $C_2H_3NaO_2$.

Sodium acetate (50 g/l) TS. A solution of sodium acetate R containing about 50 g of $C_2H_3NaO_2$ per litre.

Sodium acetate (60 g/l) TS. A solution of sodium acetate R containing about 60 g of $C_2H_3NaO_2$ per litre.

Sodium acetate R. $C_2H_3NaO_2,3H_2O$. Contains not less than 99.0% of $C_2H_3NaO_2,3H_2O$.

Description. Colourless crystals.

Solubility. Very soluble in water; sparingly soluble in ethanol (~750 g/l) TS.

Clarity and colour of solution. A 0.1 g/ml solution is clear and colourless.

pH value. pH of a 50 mg/ml solution, 7.5–9.2.

Iron. Use 8 g; the solution complies with the 2.2.4 Limit test for iron; not more than 5.0 µg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described in 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metals content according to Method A; not more than 10 µg/g.

Substances reducing permanganate. Dissolve 1 g in 100 ml of boiling water, add 2 ml of sulfuric acid (~100 g/l) TS and 0.05 ml of potassium permanganate (0.02 mol/l) VS, and boil for 5 minutes; the pink colour does not entirely disappear.

Assay. Dissolve about 0.4 g, accurately weighed, in 100 ml of glacial acetic acid R and 5 ml of acetic anhydride R. After 5 minutes add 10 drops of 1-naphtholbenzein/acetic acid TS, and titrate to a green end-point with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 13.61 mg of $C_2H_3NaO_2,3H_2O$.

Sodium acetate/glacial acetic acid (0.1 mol/l) VS

Procedure. Dissolve 5.3 g of anhydrous sodium carbonate R in small portions in 100 ml of glacial acetic acid R1, stirring well after each addition, and add sufficient glacial acetic acid R1 to produce 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution in the following manner: Titrate the solution against 15.0 ml of perchloric acid (0.1 mol/l) VS using 2–3 drops of crystal violet/acetic acid TS. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 8.203 mg of $C_2H_3NaO_2$.

Sodium alizarinsulfonate (1 g/l) TS

Procedure. Dissolve 0.11 g of sodium alizarinsulfonate R in sufficient water to produce 100 ml.

Sodium alizarinsulfonate (10 g/l) TS. A solution of sodium alizarinsulfonate R containing about 10 g of $C_{14}H_7NaO_7S$ per litre.

Sodium alizarinsulfonate R. Alizarin Red S, sodium salt of 3,4-dihydroxy-9,10-anthraquinone-2-sulfonic acid; $C_{14}H_7NaO_7S,H_2O$.

Description. A yellow-brown or orange-yellow powder.

Solubility. Freely soluble in water, producing a yellow solution; sparingly soluble in ethanol (~750 g/l) TS.

Sodium amidotrizoate RS. International Chemical Reference Substance.

Sodium arsenite (0.05 mol/l) VS

Procedure. Dissolve 5 g of arsenic trioxide R in a mixture of 20 ml of sodium hydroxide (~80 g/l) TS and 20 ml of water, dilute to 400 ml with water, and add hydrochloric acid (~70 g/l) TS until the solution is neutral to litmus paper R. Dissolve 4 g of sodium hydrogen carbonate R in the prepared solution and dilute to 1000 ml with water.

Method of standardization. Ascertain the exact concentration of the 0.05 mol/l solution in the following manner; dilute 25 ml with 50 ml of water, add 5 g of sodium hydrogen carbonate R, and titrate with iodine (0.05 mol/l) VS, using starch TS as indicator.

Storage. Add 1 drop of mercury R for the preservation of the solution.

Sodium arsenite (0.1 mol/l) VS

Procedure. Dissolve 5 g of arsenic trioxide R in a mixture of 20 ml of sodium hydroxide (~80 g/l) TS and 20 ml of water, dilute to 400 ml with water and add hydrochloric acid (~70 g/l) TS until the solution is neutral to litmus paper R. Dissolve 2 g of sodium hydrogen carbonate R in the prepared solution and dilute to 500 ml with water.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium arsenite (0.05 mol/l) VS.

Storage. Add 1 drop of mercury R for the preservation of the solution.

Sodium carbonate (10 g/l) TS. A solution of sodium carbonate R containing about 10.6 g of Na_2CO_3 per litre (approximately 0.1 mol/l).

Sodium carbonate (200 g/l) TS. A solution of sodium carbonate R containing 200 g of Na_2CO_3 per litre.

Sodium carbonate (50 g/l) TS. A solution of sodium carbonate R containing about 50 g/l of Na_2CO_3 (approximately 0.5 mol/l).

Sodium carbonate (75 g/l) TS. A solution of sodium carbonate R containing about 75 g of Na_2CO_3 per litre.

Sodium carbonate R. $Na_2CO_3,10H_2O$ (SRIP, 1963, p. 179).

Sodium carbonate standard TS

Procedure. Dissolve 2.64 g of sodium carbonate R and 2.093 g of sodium hydrogen carbonate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Sodium carbonate, anhydrous, FeR. Anhydrous sodium carbonate R that complies with the following additional test: Dissolve 4.0 g in 25 ml of water, add 8 ml of hydrochloric acid (~250 g/l) FeTS, and proceed with the 2.2.4 Limit test for iron, using 2 ml of iron standard FeTS; not more than 10 µg/g.

Sodium carbonate, anhydrous, R. Na_2CO_3 (SRIP, 1963, p. 179).

Sodium chloride (10 g/l) TS. A solution of sodium chloride R containing about 10 g of NaCl per litre.

Sodium chloride (300 g/l) TS. A solution of sodium chloride R containing about 300 g of NaCl per litre.

Sodium chloride (400 g/l) TS. A saturated solution of sodium chloride R containing about 400 g of NaCl per litre.

Sodium chloride (9 g/l) TS. A solution of sodium chloride R containing about 9 g of NaCl per litre.

Sodium chloride R. NaCl (SRIP, 1963, p. 181).

Sodium chloride, pyrogen-free, R. Sodium chloride R which complies with the following additional test:

Pyrogens. Carry out the test as described under 3.5 Test for pyrogens injecting, per kg of the rabbit's weight, a solution containing 9 mg in 10 ml of sterile water R.

Sodium citrate (250 g/l) TS. A solution of sodium citrate R containing about 294 g of $C_6H_5Na_3O_7,2H_2O$ in 1000 ml.

Sodium citrate R. $C_6H_5Na_3O_7,2H_2O$.

Contains not less than 99.0% of $C_6H_5Na_3O_7$, calculated with reference to the anhydrous substance.

Description. White, granular crystals or a crystalline powder; odourless. Slightly deliquescent in moist air.

Solubility. Soluble in less than 2 parts of water, practically insoluble in ethanol (~750 g/l) TS.

Appearance of solution. A 100 g/l solution is clear and colourless.

Water. Determined by the Karl Fischer method, keeping the substance in contact with the dehydrated methanol R for 15 minutes; not less than 110 mg/g and not more than 130 mg/g.

Assay. Dissolve about 0.15 g, accurately weighed, in 20 ml of glacial acetic acid R, and titrate with perchloric acid (0.1 mol/l) VS as described in 2.6 Non-aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 8.603 mg of $C_6H_5Na_3O_7$.

Sodium cobaltinitrite (100 g/l) TS. A solution of sodium cobaltinitrite R containing about 100 g/l of $Na_3Co(NO_2)_6$.

Sodium cobaltinitrite R. $Na_3Co(NO_2)_6$ (SRIP, 1963, p. 182).

Sodium cromoglicate RS. International Chemical Reference Substance.

Sodium diethyldithiocarbamate (0.8 g/l) TS. A solution of sodium diethyldithiocarbamate R containing about 0.8 g of $C_5H_{10}NNaS_2$ per litre.

Sodium diethyldithiocarbamate R. $C_5H_{10}NNaS_2,3H_2O$ (SRIP, 1963, p. 183).

Sodium dihydrogen phosphate (275 g/l) TS. A solution of sodium dihydrogen phosphate R containing about 275 g of NaH_2PO_4 per litre.

Sodium dihydrogen phosphate (45 g/l) TS. A solution of sodium dihydrogen phosphate R containing about 47 g of NaH_2PO_4 per litre.

Sodium dihydrogen phosphate, anhydrous R. [sodium biphosphmate]; sodium-phosphate, monobasic; NaH_2PO_4.

Sodium dihydrogen phosphate dihydrate R. [sodium biphosphate]; sodium phosphate, monobasic; $NaH_2 PO_4$, 2 H_2O.

Sodium dihydrogen phosphate R [sodium biphosphate]; sodium phosphate, monobasic; NaH_2PO_4,H_2O (SRIP, 1963, p. 178).

Sodium dithionite R. Sodium hydrosulfite, sodium sulfoxylate; $Na_2O_4S_2$.
Description. A white or greyish white, crystalline powder.
Solubility. Very soluble in water, slightly soluble in ethanol (~750 g/l) TS.
Note: Sodium dithionite R oxidizes in air.

Sodium dithionite (200 g/l) TS. A solution of sodium dithionite R containing about 200 g of $Na_2O_4S_2$ per litre.

Sodium fluoride R. NaF (SRIP, 1963, p. 183).

Sodium formate R. $CHNaO_2$.
Description. White, deliquescent granules or a crystalline powder; slight odour of formic acid.
Melting temperature. About 253 °C.

Sodium hexanesulfonate R. $C_6H_{13}NaO_3S$.
A commercially available reagent of suitable grade.

Sodium hydrogen carbonate (100 g/l) TS. A solution of sodium hydrogen carbonate R containing about 100 g of $NaHCO_3$ in 1000 ml.

Sodium hydrogen carbonate (40 g/l) TS. A solution of sodium hydrogen carbonate R containing about 42 g of $NaHCO_3$ per litre (approximately 0.5 mol/l).

Sodium hydrogen carbonate R [sodium bicarbonate]; $NaHCO_3$ (SRIP, 1963, p. 177).

Sodium hydrogen carbonate R. $NaHCO_3$ (SRIP, 1963, p. 177).

Sodium hydroxide (~150 g/l) TS. A solution of sodium hydroxide R containing about 150 g of NaOH per litre.

Sodium hydroxide (~200 g/l) TS. A solution of sodium hydroxide R containing about 200 g/l of NaOH.

Sodium hydroxide (~300 g/l) TS. A solution of sodium hydroxide R containing about 300 g/l of NaOH.

Sodium hydroxide (~400 g/l) TS. A solution of sodium hydroxide R containing about 400 g/l of NaOH.

Sodium hydroxide (~80 g/l) TS. A solution of sodium hydroxide R containing about 80 g/l of NaOH (approximately 2 mol/l).

Sodium hydroxide (~40 g/l) TS. A solution of sodium hydroxide R containing about 40 g/l of NaOH (approximately 1 mol/l).

Sodium hydroxide (0.001 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 40.01 mg of NaOH in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.01 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 0.4001 g of NaOH in 1000 ml.
Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.01 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 0.4001 g of NaOH in 1000 ml.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test, and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.02 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 0.8001 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.02 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 0.8001 g of NaOH in 1000 ml.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test, and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.05 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 2.000 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.1 mol/l) BET. Prepare from sodium hydroxide R and water BET. It is suitable if, after adjustment to pH 6.5–7.5, it gives a negative result under the conditions prescribed in the 3.4 Test for bacterial endotoxins.

Sodium hydroxide (0.1 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 4.001 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.1 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 4.001 g of NaOH in 1000 ml.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test, and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.2 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 8.001 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.2 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 8.001 g of NaOH in 1000 ml.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test, and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.5 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 20.00 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (0.5 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 20.00 g of NaOH in 1000 ml.

Procedure, test for carbonates and method of standardization. Prepare the solution, carry out the test, and ascertain the exact concentration following the method described under carbonate-free sodium hydroxide (1 mol/l) VS.

Sodium hydroxide (1 mol/l) VS. Sodium hydroxide R, dissolved in water to contain 40.01 g of NaOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: Dry about 5 g of potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals, they should be crushed before drying. Dissolve in 75 ml of carbon-dioxide-free water R and titrate with the sodium hydroxide solution, using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equivalent to 1 ml of sodium hydroxide (1 mol/l) VS. Standard solutions of sodium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should, therefore, be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Sodium hydroxide (1 mol/l), carbonate-free, VS. Sodium hydroxide R, dissolved in water to contain 40.01 g of NaOH in 1000 ml.

Procedure. Dissolve sodium hydroxide R in water to produce a 400–600 g/l solution and allow to stand. Taking precautions to avoid absorption of carbon dioxide, siphon off the clear supernatant liquid and dilute as required with carbon-dioxide-free water R.

Test for carbonates. Titrate 45 ml of hydrochloric acid (1 mol/l) VS with the carbonate-free sodium hydroxide solution, using phenolphthalein/ethanol TS as indicator. At the end-point add just sufficient acid to discharge the pink colour and boil to reduce the volume to 20 ml. Add, whilst boiling, sufficient acid again to discharge the pink colour and prevent its reappearance on continued boiling; not more than 0.1 ml of the acid is required.

Method of standardization. Ascertain the exact concentration of the 1 mol/l solution in the following manner: Dry about 5 g of potassium hydrogen phthalate R at 105 °C for 3 hours and weigh accurately. If the potassium hydrogen phthalate is in the form of large crystals, they should be crushed before drying. Dissolve in 75 ml of carbon-dioxide-free water R and titrate with the carbonate-free sodium hydroxide solution, using phenolphthalein/ethanol TS as indicator. Each 0.2042 g of potassium hydrogen phthalate is equiva-

lent to 1 ml of carbonate-free sodium hydroxide (1 mol/l) VS. Standard solutions of sodium hydroxide should be restandardized frequently.

Storage. Solutions of alkali hydroxides absorb carbon dioxide when exposed to air They should therefore be stored in bottles with suitable non-glass, well-fitting stoppers, provided with a tube filled with soda lime R.

Sodium hydroxide (10 g/l) TS. A solution of sodium hydroxide R containing about 10 g of NaOH per litre (approximately 0.25 mol/l).

Sodium hydroxide (50 g/l) TS. A solution of sodium hydroxide R containing about 50 g of NaOH per litre.

Sodium hydroxide R. NaOH (SRIP, 1963, p. 185).

Sodium hydroxide/ethanol TS

Procedure. Dissolve 50 g of sodium hydroxide R in sufficient ethanol (~750 g/l) TS to produce 1000 ml.

Sodium hydroxide/methanol TS

Procedure. Dissolve 40 g of sodium hydroxide R in sufficient methanol R to produce 1000 ml.

Sodium hypobromite TS

Procedure. Dissolve 2.5 g of sodium hydroxide R in 7.5 ml of water, add 0.5 ml of bromine R and a sufficient quantity of water to produce 10 ml.

Note: Sodium hypobromite TS must be freshly prepared.

Sodium hypochlorite (~40 g/l) TS

Description. A pale, greenish yellow, clear liquid; odour, resembling that of chlorine.

Assay. Introduce 3 ml into a glass-stoppered flask, weigh accurately, and add 50 ml of water. Add 2 g of potassium iodide R and 10 ml of acetic acid (~300 g/l) TS, and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS, adding 3 ml of starch TS as the endpoint is approached. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 3.723 mg of NaOCl.

Storage. Sodium hypochlorite (~40 g/l) TS must be kept in a light-resistant container at a temperature not exceeding 25 °C.

Sodium hypochlorite TS1

Procedure. Dilute 10 ml of sodium hypochlorite (~40 g/l) TS to 100 ml with water (contains approximately 0.5% of chlorine).

Sodium laurilsulfate (10 g/l) TS. A solution of sodium laurilsulfate R containing about 10 g of $C_{12}H_{25}NaO_4S$ per litre.

Sodium laurilsulfate R. Sodium lauryl sulfate; a mixture of sodium alkyl sulfates, consisting mainly of sodium dodecyl sulfates, $C_{12}H_{25}NaO_4S$.

Description. A white or pale yellow powder, crystals, or flakes; odour, faint, but characteristic.

Solubility. Very soluble in water giving an opalescent solution; partly soluble in ethanol (~750 g/l) TS.

Sodium mercaptoacetate R (Sodium thioglycolate R.) $C_2H_3NaO_2S$.

Description. Hygroscopic crystals.

Solubility. Freely soluble in water; slightly soluble in ethanol (~750 g/l) TS.

Sodium metabisulfite (50 g/l) TS. A solution of sodium metabisulfite R containing about 50 g of $Na_2O_5S_2$ per litre.

Sodium metabisulfite R. $Na_2O_5S_2$ (SRIP, 1963, p. 187).

Sodium metaperiodate R. Sodium periodate $NaIO_4$. Contains not less than 98.0% of $NaIO_4$.

Description. White crystals or a white, crystalline powder.

Solubility. Soluble in water.

Assay. Dissolve 0.5 g in 100 ml of water. Add 3 g of sodium hydrogen carbonate R and 3 g of potassium iodide R, and titrate the liberated iodine with sodium arsenite (0.05 mol/l) VS. Each ml of sodium arsenite (0.05 mol/l) VS is equivalent to 10.69 mg of $NaIO_4$.

Sodium metaperiodate TS

Procedure. Dissolve 60 g of sodium metaperiodate R in 120 ml of sulfuric acid (0.05 mol/l) VS and dilute to 1000 ml with water. *Do not heat to dissolve the periodate.* If the solution is not clear, filter through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container.

Suitability test. Pipette 10 ml into a 250-ml volumetric flask, dilute to volume with water, and mix. To about 550 mg of glycerol R dissolved in 50 ml of water add, using a pipette, 50 ml of the diluted sodium metaperiodate. For a blank, transfer 50 ml of the diluted sodium metaperiodate solution to a flask containing 50 ml of water. Allow the solutions to stand for 30 minutes, then to each add 5 ml of hydrochloric acid (~420 g/l) TS and 10 ml of potassium iodide (80 g/l) TS, and swirl to mix. Allow to stand for 5 minutes, add 100 ml of water, and titrate with sodium thiosulfate (0.1 mol/l) VS, shaking continuously and adding 3 ml of starch TS as the endpoint is approached. The ratio of the volume of sodium thiosulfate (0.1 mol/l) VS required for sodium metaperiodate TS to that required for the blank should be between 0.750 and 0.765.

Sodium methoxide (0.1 mol/l) VS

Procedure. Cool in ice-water 150 ml of dehydrated methanol R and add in small portions 2.5 g of freshly cut sodium R. When the metal has dissolved, add sufficient toluene R to produce 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Titrate 0.10 g of benzoic acid R, accurately weighed, as described under 2.6 Non-aqueous titration, Method B. Each 12.21 mg of $C_7H_6O_6$ is equivalent to 1 ml of sodium methoxide (0.1 mol/l) VS.

Note: Sodium methoxide (0.1 mol/l) VS must be standardized immediately before use.

Sodium molybdotungstophosphate TS

Procedure. Boil under a reflux condenser for 2 hours 350 ml of water with 50 g of sodium tungstate R, 12 g of phosphomolybdic acid R, and 25 ml of phosphoric acid (~1440 g/l) TS; cool and add sufficient water to produce 500 ml.

Sodium nitrate R. $NaNO_3$.

A commercially available reagent of suitable grade.

Sodium nitrite (0.1 mol/l) VS. Sodium nitrite R, dissolved in water to contain 6.900 g of $NaNO_2$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: Place 50.0 ml of potassium permanganate (0.02 mol/l) VS in a glass-stoppered flask, dilute with 300 ml of water, add 25 ml of sulfuric acid (~100 g/l) TS and 20.0 ml of the sodium nitrite solution. Allow the solution to stand for 10 minutes. Then add 2 g of potassium iodide R and titrate with sodium thiosulfate (0.1 mol/l) VS, using starch TS as indicator. Perform a blank determination and make any necessary corrections.

Sodium nitrite (1 g/l) TS. A solution of sodium nitrite R containing about 1 g of $NaNO_2$ per litre.

Note: Sodium nitrite (1 g/l) TS must be freshly prepared.

Sodium nitrite (10 g/l) TS. A solution of sodium nitrite R containing about 10 g/l of $NaNO_2$.

Sodium nitrite (100 g/l) TS. A solution of sodium nitrite R containing about 100 g of $NaNO_2$ per litre.

Sodium nitrite (20 g/l) TS. A solution of sodium nitrite R containing about 20 g of $NaNO_2$ per litre.

Sodium nitrite (3 g/l) TS. A solution of sodium nitrite R containing about 3 g of $NaNO_2$ per litre.

Note: Sodium nitrite (3 g/l) TS must be freshly prepared.

Sodium nitrite (35 g/l) TS. A solution of sodium nitrite R containing about 35 g of $NaNO_2$ per litre (approximately 0.5 mol/l).

Sodium nitrite (50 g/l) TS. A solution of sodium nitrite R containing about 50 g of $NaNO_2$ per litre.

Sodium nitrite R. $NaNO_2$ (SRIP, 1963, p. 189).

Sodium nitrite/hydrochloric acid TS
Procedure. Dissolve 0.5 g of sodium nitrite R in sufficient hydrochloric acid (0.1 mol/l) VS to produce 100 ml.

Sodium nitroprusside (45 g/l) TS. A solution of sodium nitroprusside R containing about 45 g of $Na_2Fe(NO)(CN)_5$ per litre.
Note: Sodium nitroprusside (45 g/l) TS must be freshly prepared.

Sodium nitroprusside (8.5 g/l) TS. A solution of sodium nitroprusside R containing about 8.5 g of $Na_2Fe(NO)(CN)_5$ per litre.

Sodium nitroprusside R. $Na_2Fe(NO)(CN)_5,2H_2O$ (SRIP, 1963, p. 190).

Sodium nitroprusside, alkaline, TS
Procedure. Dissolve 1 g of sodium nitroprusside R and 1 g of sodium carbonate R in sufficient water to produce 100 ml.

Sodium octanesulfonate R. $C_8H_{17}NaO_3S$.
Contains not less than 98.0% of $C_8H_{17}NaO_3S$.
A commercially available reagent of suitable grade.
Absorbance. A 0.05 g/ml solution of a 1-cm layer measured at a wavelength of about 250 nm has an absorbance of not greater than 0.01.

Sodium oxalate R. $C_2Na_2O_4$ (SRIP, 1963, p. 190).

Sodium peroxide R. Na_2O_2 (SRIP, 1963, p. 191).

Sodium R. Na (SRIP, 1963, p. 175).

Sodium salicyclate R. $C_7H_5NaO_3$. Use sodium salicylate as described in the monograph for "Sodium salicylate".

Sodium salicylate (11.5 g/l) TS. A solution of sodium salicylate R containing about 11.5 g of $C_7H_5NaO_3$ per litre.

Sodium standard (200 µg Na/ml) TS
Procedure. Dissolve 0.5084 g of sodium chloride R, previously dried at 100–105 °C for 3 hours, in sufficient water to produce 1000 ml.

Sodium sulfate, anhydrous, R. Na_2SO_4 (SRIP, 1963, p. 195).

Sodium sulfide R. $Na_2S,9H_2O$ (SRIP, 1963, p. 195).

Sodium sulfide TS

Procedure. Dissolve 12 g of sodium sulfide R in 25 ml of water and add sufficient glycerol R to produce 100 ml.

Sodium sulfite R. $Na_2SO_3,7H_2O$ (SRIP, 1963, p. 196).

Sodium tetraborate (10 g/l) TS. A solution of sodium tetraborate R containing about 10 g of $Na_2B_4O_7$ per litre.

Sodium tetraborate R. Borax, $Na_2B_4O_7,10H_2O$.

Description. Transparent, colourless crystals, or a white, crystalline powder; odourless.

Solubility. Soluble in 20 parts of water, and in 0.6 part of boiling water; very slightly soluble in ethanol (~750 g/l) TS.

pH Value of a 0.01 mol/l solution. Dissolve 0.3814 g in water and dilute to 100 ml, using water having a pH of 6.5–7.4. The pH should be from 9.15–9.20 at 25 °C.

Chlorides. Dissolve 1.0 g in 20 ml of water, filter if necessary through a chloride-free filter, add 1 ml of nitric acid (~1000 g/l) TS, and proceed as described in 2.2.1 Limit test for chlorides. Sodium tetraborate R contains not more than 250 µg/g.

Sulfates. Dissolve 0.5 g in 20 ml of water, add 2 ml of hydrochloric acid (~70 g/l) TS, and filter. Proceed as described in 2.2.2 Limit test for sulfates. Sodium tetraborate R contains not more than 1.0 mg/g.

Sodium tetraborate standard TS

Procedure. Dissolve 3.81 g of sodium tetraborate R in sufficient carbon-dioxide-free water R to produce 1000 ml.

Storage. Store the solution protected from atmospheric carbon dioxide and keep it stoppered at all times except when actually in use.

Sodium tetraphenylborate (30 g/l) TS. A solution of sodium tetraphenylborate R containing about 30 g/l of $C_{24}H_{20}BNa$.

Note: If necessary, stir for 5 minutes with 1 g of aluminium hydroxide R or charcoal R, and filter to clarify.

Sodium thioglycolate R. *See* Sodium mercaptoacetate R.

Sodium tetraphenylborate R. $C_{24}H_{20}BNa$.

Description. A fluffy, white or almost white powder.

Solubility. Freely soluble in water and acetone R; insoluble in light petroleum R.

pH Value. pH of a 20 g/l solution, not less than 7.5.

Sodium thiosulfate (0.002 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 0.316 g of $Na_2S_2O_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium thiosulfate (0.1 mol/l) VS.

Sodium thiosulfate (0.01 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 1.582 g of $Na_2S_2O_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under sodium thiosulfate (0.1 mol/l) VS.

Sodium thiosulfate (0.02 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 3.164 g of $Na_2S_2O_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium thiosulfate (0.1 mol/l) VS.

Sodium thiosulfate (0.05 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 7.910 g of $Na_2S_2O_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under sodium thiosulfate (0.1 mol/l) VS.

Sodium thiosulfate (0.1 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 15.82 g of $Na_2S_2O_3$ in 1000 ml.

Method of standardization (alternative procedure). Ascertain the exact concentration of the 0.1 mol/l solution in the following manner: To about 40 ml of water in a glass-stoppered conical flask, add 10.0 ml of potassium bromate (0.0167 mol/l) VS, 1 g of potassium iodide R, and 3 ml of sulfuric acid (~1760 g/l) TS. Allow the solution to stand for 5 minutes, and titrate the liberated iodine with the sodium thiosulfate solution, adding 3 ml of starch TS as the end-point is approached. Perform a blank determination on the same quantities of the reagents and make any necessary corrections.

Sodium thiosulfate (320 g/l) TS. A solution of sodium thiosulfate R containing about 320 g of $Na_2S_2O_3$ per litre.

Sodium thiosulfate R. $Na_2S_2O_3,5H_2O$ (SRIP, 1963, p. 197).

Sodium tungstate R. $Na_2O_4W,2H_2O$ (SRIP, 1963, p. 197).

Sorbitol R. $C_6H_{14}O_6$. Contains not less than 97.0% of $C_6H_{14}O_6$.

Description. White granules or powder or a white, crystalline mass.

Solubility. Very soluble in water; sparingly soluble in ethanol (~750 g/l) TS; practically insoluble in ether R.

Assay. Dissolve about 0.2 g, previously dried and accurately weighed, in sufficient water to produce 100 ml. Transfer 10.0 ml to an iodine flask, add 50.0 ml of potassium periodate TS, and heat for 15 minutes on a water-bath. Cool, add 2.5 g of potassium iodide R, stopper tightly, and shake well. Allow

to stand for 5 minutes protected from light and titrate with sodium thiosulfate (0.1 mol/l) VS, using 3 ml of starch TS as an indicator. Perform a blank titration and make any necessary corrections. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 1.822 mg of $C_6H_{14}O_6$.

Storage. Store in a tightly closed container.

Spectinomycin hydrochloride RS. International Chemical Reference Substance.

Spironolactone RS. International Chemical Reference Substance.

Squalane R. 2,6,10,15,19,23-Hexamethyltetracosane; $C_{30}H_{62}$.

Description. A colourless, oily liquid.

Solubility. Freely soluble in ether R; slightly soluble in acetone R and ethanol (~750 g/l) TS.

Relative density. $d_{20}^{20} = 0.811–0.813$.

Refractive index. $n_D^{20} = 1.451–1.453$.

Stannous chloride AsTS

Procedure. Prepare from stannous chloride TS by adding an equal volume of hydrochloric acid (~250 g/l) TS, boil down to the original volume, and filter through a fine-grained filter-paper.

Test for arsenic. To 10 ml add 6 ml of water and 10 ml of hydrochloric acid (~250 g/l) AsTS, and distil 16 ml. To the distillate add 50 ml of water and 2 drops of stannous chloride AsTS; then apply the general test for arsenic. The colour of the stain produced is not more intense than that produced from a 1-ml standard stain, showing that the amount of arsenic does not exceed 1 µg/ml.

Stannous chloride R. $SnCl_2,2H_2O$ (SRIP, 1963, p. 198).

Stannous chloride TS

Procedure. Dissolve 330 g of stannous chloride R in 100 ml of hydrochloric acid (~250 g/l) TS and sufficient water to produce 1000 ml.

Stannous chloride/hydrochloric acid TS1

Procedure. Dissolve 10 g of stannous chloride R in sufficient hydrochloric acid (~70 g/l) TS to produce 100 ml.

Starch iodide TS

Procedure. Dissolve 0.75 g of potassium iodide R in 5 ml of water and 2 g of zinc chloride R in 10 ml of water, mix the two solutions, and add 100 ml of water. Heat the solution to boiling and add, with constant stirring, a suspension of 5 g of corn or potato starch R in 35 ml of water. Boil for 2 minutes and cool.

Storage. Store in a well-closed container and keep in a cool place.

Starch R [potato starch R or corn starch R]. (SRIP, 1963, p. 199).

Starch TS
Procedure. Mix 0.5 g of starch R or of soluble starch R with 5 ml of water, and add this solution, with constant stirring, to sufficient water to produce about 100 ml; boil for a few minutes, cool, and filter.
Note: Starch TS must be freshly prepared.

Starch, soluble, R. (SRIP, 1963, p. 199).

Starch/iodide paper R [starch-iodide paper R]. (SRIP, 1963, p. 200).

Streptomycin sulfate RS. International Chemical Reference Substance.

Strychnine sulfate R. $C_{42}H_{44}N_4O_4,H_2SO_4,5H_2O$ (SRIP, 1963, p. 200).

Sudan red G R. 1-(4-Phenylazophenylazo)-2-naphthol; Sudan III; Solvent red 23; C.I. 26100; $C_{22}H_{16}N_4O$.
Description. A reddish brown powder.
Solubility. Practically insoluble in water.

Sudan red TS
Procedure. Dissolve 0.5 g of sudan red G R in 100 ml of glacial acetic acid R1.

Sulfacetamide RS. International Chemical Reference Substance.

Sulfadiazine RS. International Chemical Reference Substance.

Sulfadimidine RS. International Chemical Reference Substance.

Sulfadoxine RS. International Chemical Reference Substance.

Sulfamethoxazole RS. International Chemical Reference Substance.

Sulfamethoxypyridazine RS. International Chemical Reference Substance.

Sulfamic acid (5 g/l) TS. A solution of sulfamic acid R containing about 5 g of H_3NO_3S per litre.

Sulfamic acid (50 g/l) TS. A solution of sulfamic acid R containing about 50 g of H_3NO_3S per litre.
Note: Sulfamic acid (50 g/l) TS must be freshly prepared.

Sulfamic acid (80 g/l) TS. A solution of sulfamic acid R containing about 80 g of H_3NO_3S per litre.
Note: Sulfamic acid (80 g/l) TS must be freshly prepared.

Sulfamic acid R. H_3NO_3S.
Description. Colourless or white crystals.
Solubility. Soluble in water; slightly soluble in ethanol (~750 g/l) TS.

4-Sulfamoylbenzoic acid R. *p*-Sulfamoylbenzoic acid; $C_7H_7NO_4S$.
Melting point. About 291 °C.

Sulfanilamide RS. International Chemical Reference Substance.

Sulfanilic acid R. $C_6H_7NO_3S$ (SRIP, 1963, p. 201).

Sulfanilic acid, diazotized, TS
Procedure. Dissolve 0.2 g of sulfanilic acid R in 20 ml of hydrochloric acid (1 mol/l) VS with warming, cool in ice, add drop by drop and with continuous stirring 2.5 ml of sodium nitrite (35 g/l) TS, allow to stand in ice for 10 minutes and then add 1 ml of sulfamic acid (50 g/l) TS.

Sulfasalazine RS. International Chemical Reference Substance.

Sulfosalicylic acid (175 g/l) TS. A solution of sulfosalicylic acid R containing about 175 g/l of $C_7H_6O_6S$.

Sulfosalicylic acid R. $C_7H_6O_6S,2H_2O$.
Description. White or slightly pink coloured, needle-like crystals.
Solubility. Soluble in water and ethanol (~750 g/l) TS.
Insoluble matter. Dissolve 5.0 g in 50 ml of water, heat to boiling and digest in a covered beaker on a water-bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105 °C. The weight of the residue does not exceed 1.0 mg.
Sulfated ash. Gently ignite 1.0 g in a tared crucible or dish, other than platinum, until charred. Cool, moisten the residue with 1 ml of sulfuric acid (~1760 g/l) TS, and ignite again; not more than 1.0 mg/g.

Sulfur dioxide R. SO_2 (SRIP, 1963, p. 202).

Sulfuric acid (~10 g/l) TS
Procedure. Mix 100 ml of sulfuric acid (~100 g/l) TS with sufficient water to produce 1000 ml.

Sulfuric acid (~100 g/l) TS
Procedure. Add 57 ml of sulfuric acid (~1760 g/l) TS to sufficient water to produce 1000 ml (approximately 1 mol/l); $d \sim 1.065$.

Sulfuric acid (~1125 g/l) TS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain about 1125 g of H_2SO_4 per litre; $d \sim 1.61$.

Sulfuric acid (~1760 g/l) TS [sulfuric acid R]. (SRIP, 1963, p. 202); *d* ~ 1.84.

Sulfuric acid (~1760 g/l), nitrogen-free, TS. Sulfuric acid (~1760 g/l) TS containing not less than 1760 g/l of H_2SO_4 and complying with the test for nitrates.

Nitrates. Mix 45 ml with 5 ml of water, cool, and add 8 mg of diphenylbenzidine R; the solution is colourless or not more than very pale blue.

Sulfuric acid (~190 g/l) TS

Procedure. Mix 1 volume of sulfuric acid (~1760 g/l) TS with 9 volumes of water, and cool. The resulting solution contains about 190 g/l of H_2SO_4; *d* ~ 1.12.

Sulfuric acid (~440 g/l) TS

Procedure. Dilute 485 ml of sulfuric acid (~1760 g/l) TS to 1000 ml with water (~4.5 mol/l); *d* ~ 1.25.

Sulfuric acid (~50 g/l) TS

Procedure. To 50 ml of sulfuric acid (~100 g/l) TS add about 50 ml of water and mix.

Sulfuric acid (~570 g/l) TS

Procedure. Slowly add 3 volumes of sulfuric acid (~1760 g/l) TS to 7 volumes of water while gently stirring and cool; *d* ~ 1.33.

Sulfuric acid (~635 g/l) TS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain about 635 g of H_2SO_4 per litre; *d* ~ 1.36.

Sulfuric acid (~700 g/l) TS

Procedure. Slowly add sulfuric acid (~1760 g/l) TS to an equal weight of water while gently stirring and cool; *d* ~ 1.40.

Sulfuric acid (0.005 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 0.4904 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.01 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 0.9808 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.05 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 4.904 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.1 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 9.808 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.125 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 12.52 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.25 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 24.52 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sulfuric acid (0.5 mol/l) VS.

Sulfuric acid (0.5 mol/l) VS. Sulfuric acid (~1760 g/l) TS, diluted with water to contain 49.04 g of H_2SO_4 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the 0.5 mol/l solution in the following manner: Dissolve about 1.5 g, accurately weighed, of anhydrous sodium carbonate R, previously dried at 270 °C for 1 hour, in 50 ml of water and titrate with the sulfuric acid solution, using methyl orange/ethanol TS as indicator. Each 52.99 mg of anhydrous sodium carbonate is equivalent to 1 ml of sulfuric acid (0.5 mol/l) VS.

Sulfuric acid/ethanol TS

Procedure. Cool separately 10 ml of ethanol (~750 g/l) TS and 90 ml of sulfuric acid (~1760 g/l) TS to about −5 °C. Carefully add the acid to the ethanol, keeping the solution as cool as possible, and mix gently.

Sulfuric acid/methanol TS

Procedure. Cool separately 10 ml of sulfuric acid (~1760 g/l) TS and 90 ml of methanol R. Carefully add the acid to the methanol, keeping the solution as cool as possible, and mix gently.

Sulfurous acid TS [sulfurous acid R]. (SRIP, 1963, p. 204).

Tamoxifen citrate E-isomer RS. International Chemical Reference Substance.

Tamoxifen citrate RS. International Chemical Reference Substance.

Tannic acid (50 g/l) TS. A solution of tannic acid R containing about 50 g of $C_{76}H_{52}O_{46}$ per litre.

Tannic acid R. $C_{76}H_{52}O_{46}$ (SRIP, 1963, p. 205).

Tartaric acid (10 g/l) TS. A solution of tartaric acid R containing about 10 g of $C_4H_6O_6$ per litre.

Tartaric acid (200 g/l) TS. A solution of tartaric acid R containing about 200 g of $C_4H_6O_6$ per litre.

Tartaric acid (5 g/l) TS. A solution of tartaric acid R containing about 5 g of $C_4H_6O_6$ per litre.

Tartaric acid R. $C_4H_6O_6$ (SRIP, 1963, p. 205).

p-Terphenyl R. 1,4-Diphenylbenzene, $C_{18}H_{14}$. Suitable for scintillation counting.

Thioglycolic acid R. *See* **Mercaptoacetic acid R.**

Testosterone enantate RS. International Chemical Reference Substance.

Testosterone propionate R. $C_{22}H_{32}O_3$. Use testosterone propionate as described in the monograph for "Testosterone propionate".

Testosterone propionate RS. International Chemical Reference Substance.

Testosterone propionate/ethanol TS

Procedure. Dissolve 10 mg of testosterone propionate R in sufficient ethanol (~750 g/l) TS to produce 10 ml.

Tetrabromophenolphthalein ethyl ester R. 3′,3″,5′,5″-Tetrabromophenolphthalein, ethyl ester; $C_{22}H_{14}Br_4O_4$. Use a suitable reagent grade.

Tetrabromophenolphthalein ethyl ester TS

Procedure. Dissolve 0.10 g of tetrabromophenolphthalein ethyl ester R in sufficient glacial acetic acid R to produce 100 ml.

Note: Tetrabromophenolphthalein ethyl ester TS should be freshly prepared.

Tetrabutylammonium hydrogen sulfate R. TBAHS; $C_{16}H_{37}NO_4S$.

A commercially available reagent of suitable grade.

Description. Colourless crystals or a white, crystalline powder.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/l) TS producing a slightly hazy, colourless solution.

Absorbance. A 0.05 g/ml solution of a 1-cm layer measured at a wavelength between 240 nm and 300 nm has an absorbance of not greater than 0.05.

Melting range. 169–173 °C.

Tetrabutylammonium hydroxide (0.1 mol/l) VS

Procedure. Dissolve 40 g of tetrabutylammonium iodide R in 90 ml of dehydrated ethanol R, add 20 g of finely powdered, purified silver oxide R, and

shake vigorously for 1 hour. Centrifuge a small volume of the mixture and test the supernatant liquid for iodides. If a positive reaction is obtained, add an additional 2 g of silver oxide R and shake for a further 30 minutes. Repeat this procedure until the liquid is free from iodides, filter the mixture through a fine sintered glass filter, and rinse the reaction vessel and the filter with 3 quantities of dry benzene R, each of 50 ml. Add the washings to the filtrate and dilute to 1000 ml with dry benzene R. Pass dry carbon-dioxide-free nitrogen R through the solution for 5 minutes.

Method of standardization. Titrate 10 ml of dimethylformamide R with the tetrabutylammonium hydroxide solution, using 3 drops of thymol blue/methanol TS as indicator, until a pure blue colour is obtained. Immediately add about 0.06 g of benzoic acid R, accurately weighed, stir to effect solution, and titrate with the tetrabutylammonium hydroxide solution until the full blue colour of the indicator is again obtained. The solution must be protected from atmospheric carbon dioxide throughout the titration. From the volume of the titrant used in the second titration ascertain the exact concentration of the 0.1 mol/l solution. Each 12.21 mg of benzoic acid is equivalent to 1 ml of tetrabutylammonium hydroxide (0.1 mol/l) VS.

Note: Tetrabutylammonium hydroxide (0.1 mol/l) VS must be standardized immediately before use.

Tetrabutylammonium hydroxide TS. $C_{16}H_{37}NO$. A solution in water containing about 400 g of $C_{16}H_{37}NO$ per litre (~1.5 mol/l).

Tetrabutylammonium hydroxide/methanol TS

Procedure. Dilute a sufficient volume of tetrabutylammonium hydroxide TS with methanol R to obtain a solution containing 0.25 g of $C_{16}H_{37}NO$ per ml.

Tetrabutylammonium iodide R. $C_{16}H_{36}IN$. Contains not less than 98.0% of $C_{16}H_{36}IN$.

Description. White or slightly cream-coloured crystals or a crystalline powder.

Solubility. Soluble in ethanol (~750 g/l) TS.

Sulfated ash. Not more than 0.2 mg/g.

Assay. Dissolve about 1.2 g, accurately weighed, in 30 ml of water. Add 50 ml of silver nitrate (0.1 mol/l) VS and 5 ml of nitric acid (~130 g/l) TS. Titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Each ml of silver nitrate (0.1 mol/l) VS is equivalent to 36.94 mg of $C_{16}H_{36}IN$.

n-Tetradecane R. $C_{14}H_{30}$.

Description. A clear and colourless liquid.

Miscibility. Miscible with ethanol (~750 g/l) TS.

Mass density. ρ_{20} = about 0.76 kg/l.

Refractive index. n_D^{20} = 1.428–1.429.

Tetrachloroethane R. 1,1,2,2-Tetrachloroethane, $C_2H_2Cl_4$.
Description. A clear, colourless liquid.
Miscibility. Miscible with 400 parts of water; miscible with ethanol (~750 g/l) TS and ether R.
Boiling range. Not less than 95% distils between 142 and 147 °C.
Refractive index. ρ_{20} = 1.493–1.495.
Mass density. n_D^{20} = 1.590–1.595 kg/l.

Tetracycline hydrochloride RS. International Chemical Reference Substance.

Tetrahydrofuran R. C_4H_8O.
Description. A colourless liquid; odour, characteristic, pungent.
Boiling point. About 66 °C.
Mass density. ρ_{20} = 0.884–0.886 kg/l.
Storage. Store in small, well-filled containers, protected from light.
Labelling. The name and concentration of any suitable preservative, not exceeding 0.1%, should be stated on the label.

Tetramethylammonium hydroxide (~100 g/l) TS. Contains about 100 g/l of $(CH_3)_4NOH$ in water.
Description. A clear and colourless liquid; odour, strong, ammonia-like.
Residue on evaporation. Evaporate 5 ml on a water-bath and dry at 105 °C for 1 hour; it leaves a residue of not more than 1.0 mg (0.2 mg/g).
Ammonia and other amines. Weigh accurately a quantity of the solution, equivalent to about 0.3 g of $(CH_3)_4NOH$, in a low-form weighing bottle tared with 5 ml of water. Add a slight excess of hydrochloric acid (1 mol/l) VS (about 4 ml), evaporate to dryness on a water-bath, and dry at 105 °C for 2 hours. The weight of the residue obtained, multiplied by 0.8317, represents the quantity in mg of $(CH_3)_4NOH$ corresponding to within ± 0.2% of that found in the assay.
Assay. Weigh accurately a glass-stoppered flask containing about 15 ml of water. Add a quantity of the solution equivalent to about 0.2 g of $(CH_3)_4NOH$, and weigh again. Add methyl red/ethanol TS and titrate with hydrochloric acid (0.1 mol/l) VS. Each ml of hydrochloric acid (0.1 mol/l) VS is equivalent to 9.115 mg of $(CH_3)_4NOH$.
Storage. Store in a tightly closed container.

Tetramethylammonium hydroxide/ethanol TS
Procedure. Dilute 10 ml of tetramethylammonium hydroxide (~100 g/l) TS with sufficient ethanol (~750 g/l) TS to produce 100 ml.

Thioacetamide R. C_2H_5NS.
Note: Thioacetamide R is toxic.
Description. Colourless crystals or a white, crystalline powder; odour, faint, of hydrogen sulfide.

Solubility. Freely soluble in water and ethanol (~750 g/l) TS.
Melting point. About 113 °C.

Thioacetamide, alkaline, TS
Procedure. Dissolve 0.4 g of thioacetamide R in 10 ml of water. Immediately before use add 0.2 ml of this solution to 1 ml of a mixture of 15 ml of sodium hydroxide (1 mol/l) VS, 5 ml of water, and 20 ml of glycerol R. Heat on a water-bath for 20 seconds.

Thioacetazone RS. International Chemical Reference Substance.

4,4′-Thiodianiline RS. International Chemical Reference Substance.
Note: 4,4′-Thiodianiline RS decomposes on storage, especially on exposure to the air. If a slight decomposition is suspected, the substance can be purified by treatment of a solution in methanol R with activated charcoal.

Thiopental RS. International Chemical Reference Substance.

Thiourea (0.1 g/l) TS. A solution of thiourea R containing 0.1 g of CH_4N_2S per litre.

Thiourea R. CH_4N_2S (SRIP, 1963, p. 207).

Thorin (2 g/l) TS
Procedure. Dissolve 0.2 g of thorin R in sufficient water to produce 100 ml.
Storage. Store the solution protected from light.
Shelf-life. Use within 1 week of preparation.

Thorin R. 2,7-Disodium 4-[(*o*-arsonophenyl)azo]-3-hydroxy-2,7-naphthalenedisulfonate, $C_{16}H_{11}AsN_2Na_2O_{10}S_2$.

Thorium nitrate (0.005 mol/l) VS. Thorium nitrate R, dissolved in water to contain 2.401 g of $Th(NO_3)_4$ in 1000 ml.
Method of standardization. Ascertain the exact concentration of the 0.005 mol/l solution in the following manner: Transfer 0.050 g, accurately weighed, of sodium fluoride R, previously dried, to a flask and dissolve in sufficient water to produce 250 ml. To 20.0 ml of this solution add 0.6 ml of sodium alizarinsulfonate (1 g/l) TS and then, by drops, sodium hydroxide (0.1 mol/l) VS until the colour changes from pink to yellow. Add 5 ml of acetate buffer, pH 3.0, TS and titrate with the thorium nitrate solution until the yellow colour changes to pinkish yellow. Each 0.8398 mg of sodium fluoride is equivalent to 1 ml of thorium nitrate (0.005 mol/l) VS.

Thorium nitrate R. $Th(NO_3)_4,4H_2O$.
Description. White, slightly deliquescent crystals.
Solubility. Very soluble in water and ethanol (~750 g/l) TS.

Thymol blue R. Thymolsulfonphthalein, $C_{27}H_{30}O_5S$ (SRIP, 1963, p. 207).

Thymol blue/dimethylformamide TS
Procedure. Dissolve 0.3 g of thymol blue R in sufficient dimethylformamide R to produce 100 ml.

Thymol blue/ethanol TS
Procedure. Dissolve 0.1 g of thymol blue R in sufficient ethanol (~750 g/l) TS to produce 100 ml; filter if necessary.

Thymol blue/methanol TS
Procedure. Dissolve 0.3 g of thymol blue R in sufficient methanol R to produce 100 ml.

Thymol R. $C_{10}H_{14}O$.
Description. Colourless, often large crystals, or a white, crystalline powder; odour, aromatic, resembling that of thyme.
Solubility. Soluble in about 1000 parts of water, in 1 part of ethanol (~750 g/l) TS, and in 1.5 parts of ether R.
Melting range. Between 48 and 51 °C; when the melted substance is cooled, it remains liquid at a considerably lower temperature.
Residue on volatilization. Volatilize 2 g on a water-bath and dry to constant weight at 105 °C; it leaves a residue of not more than 0.5 mg/g.
Storage. Store in tightly closed containers, protected from light.

Thymol TS1
Procedure. Dissolve 0.225 g of thymol R in sufficient carbon tetrachloride R to produce 100 ml.

Thymol TS2
Procedure. Dilute 10 ml of thymol TS1 to 100 ml with carbon tetrachloride R.

Thymol TS3
Procedure. Dilute 10 ml of thymol TS1 to 150 ml with carbon tetrachloride R.

Thymolphthalein R. $C_{28}H_{30}O_4$ (SRIP, 1963, p. 207).

Thymolphthalein/dimethylformamide TS
Procedure. Dissolve 0.1 g of thymolphthalein R in sufficient dimethylformamide R to produce 100 ml.

Thymolphthalein/ethanol TS
Procedure. Dissolve 0.1 g of thymolphthalein R in 100 ml of ethanol (~750 g/l) TS, and filter if necessary.

Tiabendazole RS. International Chemical Reference Substance.

Timolol maleate RS. International Chemical Reference Substance.

Titan yellow R. $C_{28}H_{19}N_5Na_2O_6S_4$ (SRIP, 1963, p. 208).

Titan yellow TS
Procedure. Dissolve 0.05 g of titan yellow R in sufficient water to produce 100 ml.

Titanium dioxide R. TiO_2.
Description. A white powder; odourless.
Solubility. Practically insoluble in water; slowly soluble, when heated, in sulfuric acid (~1760 g/l) TS.

Titanium dioxide/sulfuric acid TS
Procedure. To 0.1 g of titanium dioxide R add 100 ml of sulfuric acid (~1760 g/l) TS. Heat cautiously with occasional stirring until a clear solution is effected and fumes are evolved; cool.
Storage. Store in glass-stoppered bottles.

Titanium trichloride (0.1 mol/l) VS
Procedure. Dilute 100 ml of titanium trichloride R with 200 ml of hydrochloric acid (~250 g/l) TS and add sufficient carbon-dioxide-free water R to produce 1000 ml.
Method of standardization. Ascertain the exact concentration immediately before use. With the solution titrate 25 ml of ferric ammonium sulfate (0.1 mol/l) VS acidified with sulfuric acid (~100 g/l) TS in an atmosphere of carbon dioxide R, adding ammonium thiocyanate (75 g/l) TS just before the end-point as indicator. Each ml of ferric ammonium sulfate (0.1 mol/l) VS is equivalent to 15.43 mg of $TiCl_3$.

Titanium trichloride R. A solution of titanium trichloride containing about 15% of $TiCl_3$ (SRIP, 1963, p. 208).
Mass density. ρ_{20} = ~1.2 kg/l.

Tolbutamide RS. International Chemical Reference Substance.

Toluene R. C_7H_8 (SRIP, 1963, p. 209).

Toluene-2-sulfonamide RS. International Chemical Reference Substance.

4-Toluenesulfonamide R. $C_7H_9NO_2S$.
Melting range. 135–137 °C.

4-Toluenesulfonic acid R. $C_7H_8O_3S,H_2O$. Contains not less than 98.0% of $C_7H_8O_3S$.
Description. Colourless crystals or a white, crystalline powder.

Solubility. Soluble in water, ethanol (~750 g/l) TS and ether R.
Melting range. 100–105 °C.
Sulfated ash. Not more than 1.0 mg/g.
Assay. Dissolve 0.8 g, accurately weighed, in 50 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 19.02 mg of $C_7H_8O_3S,H_2O$.

4-Toluenesulfonic acid/ethanol TS
Procedure. Dissolve 20 g of 4-toluenesulfonic acid R in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Tosylchloramide sodium (15 g/l) TS. A solution of tosylchloramide sodium R containing about 16 g of $C_7H_7ClNNaO_2S$ per litre.

Tosylchloramide sodium R. $C_7H_7ClNNaO_2S,3H_2O$. Contains not less than 98.0% of $C_7H_7ClNNaO_2S,3H_2O$.
Description. White crystals or a white, crystalline powder; odour, resembling that of chlorine.
Solubility. Soluble in 7 parts of water and in 2 parts of boiling water; soluble in ethanol (~750 g/l) TS; insoluble in ether R.
Sodium chloride. Treat 1.0 g with 15 ml of dehydrated ethanol R without the aid of heat, and filter; it leaves a residue of not more than 15 mg.
Assay. Dissolve 0.4 g, accurately weighed, in 50 ml of water, placed in a glass-stoppered vessel. Add 10 ml of potassium iodide (80 g/l) TS and 5 ml of sulfuric acid (~100 g/l) TS. Allow to stand for 10 minutes and titrate the liberated iodine with sodium thiosulfate (0.1 mol/l) VS. Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 14.08 mg of $C_7H_7ClNNaO_2S,3H_2O$.
Note: Tosylychloramide sodium R is efflorescent.

Tributyl phosphate R. $C_{12}H_{27}O_4P$.
Description. A clear, colourless liquid.
Miscibility. Slightly miscible with water; miscible with most organic solvents.
Mass density. ρ_{20} = about 0.98 kg/l.
Note: Before use, wash the reagent three times as follows: Shake 60 ml with 10 ml of a solution containing 1 g of sodium chloride R and 0.1 g of disodium hydrogen phosphate R.

Trichloroacetic acid R. $C_2HCl_3O_2$. (SRIP, 1963, p. 209).

Trichloroethylene R. C_2HCl_3.
Description. A colourless or pale blue, clear, mobile liquid; odour, characteristic, resembling that of chloroform.
Miscibility. Almost immiscible with water; miscible with dehydrated ethanol R, and ether R.

Trichlorotrifluoroethane R. 1,1,2-Trichloro-1,2,2-trifluoroethane. $C_2Cl_3F_3$.
Description. A colourless, volatile liquid.
Miscibility. Immiscible with water; miscible with acetone R and ether R.

Trichlorotrifluoroethane TS
Procedure. Mix 0.05 µl of trichlorotrifluoroethane R with 1.0 ml of dichloromethane R (other suitable solvents can be used).

Triethylamine R. $C_6H_{15}N$.
Description. A colourless liquid; odour, ammoniacal.
Boiling range. 89–90 °C.
Mass density. ρ_{20} = about 0.73 kg/l.
Refractive index. n_D^{20} = 1.4003.

Triethylenediamine R. 1,4-Diazabicyclo[2.2.2]octane; $C_6H_{12}N_2$.
Description. Hygroscopic crystals.
Melting temperature. About 158 °C.
Storage. Store in a tightly closed container.

Trihexyphenidyl hydrochloride RS. International Chemical Reference Substance.

Triketohydrindene hydrate (1 g/l) TS. A solution of triketohydrindene hydrate R containing about 1 g of $C_9H_4O_3$ per litre.

Triketohydrindene hydrate R. Ninhydrin, $C_9H_4O_3,H_2O$ (SRIP, 1963, p. 210).

Triketohydrindene/butanol TS
Procedure. Dissolve 0.1 g of triketohydrindene hydrate R in sufficient 1-butanol R previously saturated with water to produce 100 ml.

Triketohydrindene/butanol/acetic acid TS
Procedure. Prepare a 20 mg/ml solution of triketohydrindene hydrate R in a mixture of 95 volumes of 1-butanol R and 5 volumes of acetic acid (~120 g/l) TS.

Triketohydrindene/cadmium TS
Procedure. Dissolve 0.050 g of cadmium acetate R in a mixture of 5 ml of water and 1 ml of glacial acetic acid R and add sufficient ethylmethylketone R to produce 50 ml. Dissolve 20 mg of triketohydrindene hydrate R in 10 ml of this solution.
Note: Prepare immediately before use.

Triketohydrindene/ethanol TS
Procedure. Prepare a saturated solution of triketohydrindene hydrate R in ethanol (~750 g/l) TS.

Triketohydrindene/methanol TS
Procedure. Dissolve 1.0 g of triketohydrindene hydrate R in sufficient methanol R to produce 100 ml.
Note. Triketohydrindene/methanol TS must be freshly prepared.

Triketohydrindene/pyridine/acetone TS
Procedure. Dissolve 0.25 g of triketohydrindene hydrate R in 100 ml of a mixture of equal volumes of pyridine R and acetone R.

Triketohydrindene/pyridine/butanol TS
Procedure. Dissolve 1 g of triketohydrindene hydrate R in 1 ml of pyridine R and dilute with sufficient 1-butanol R to produce 100 ml.
Note: It should be freshly prepared.

Triketohydrindene/sodium metabisulfite TS
Procedure. Dissolve 3 g of triketohydrindene hydrate R in 100 ml of a solution containing 4.55 g of sodium metabisulfite R in 100 ml of water.

Triketohydrindene/stannous chloride TS
Procedure. Dissolve 4 g of triketohydrindene hydrate R in 100 ml of ethylene glycol monomethyl ether R. Shake gently with 1 g of cation exchange resin (300 µm – 840 µm) and filter (solution A). Dissolve 0.16 g of stannous chloride R in 100 ml of acetate buffer, pH 5.5, TS (solution B). Immediately before use, mix equal volumes of the two solutions.

Trimethadione RS. International Chemical Reference Substance.

Trimethoprim RS. International Chemical Reference Substance.

2,2,4-Trimethylpentane R. *iso*-Octane; C_8H_{18} (SRIP, 1963, p. 129).

Trimethylpyridine (50 g/l) TS. A solution of trimethylpyridine R containing about 50 g of $C_8H_{11}N$ per litre.

Trimethylpyridine R. $C_8H_{11}N$. 2,4,6-Trimethylpyridine; sym. collidine.
Description. Liquid; odour, aromatic.
Miscibility. More miscible with cold water than with hot water; miscible with ethanol (~750 g/l) TS; ether R, and methanol R.
Relative density. $d_4^{20} = 0.914$.
Refractive index. $n_D^{20} = 1.498$.

Trinitrophenol (7 g/l) TS. A solution of trinitrophenol R containing 7 g of $C_6H_3N_3O_7$ per litre.

Trinitrophenol R. $C_6H_3N_3O_7$ (SRIP, 1963, p. 211).

Trinitrophenol, alkaline, TS
Procedure. Mix 20 ml of a 10 mg/ml solution of trinitrophenol R with 10 ml of a 50 mg/ml solution of sodium hydroxide R, dilute with water to 100 ml, and mix.
Note: After preparation alkaline trinitrophenol TS should be used for only 48 hours.

Trinitrophenol/ethanol TS
Procedure. Dissolve 33 g of trinitrophenol R in sufficient ethanol (~750 g/l) TS to produce 1000 ml.

Triphenylantimony R. $C_{18}H_{15}Sb$.
Melting temperature. About 55 °C.

Trisodium orthophosphate (2 g/l) TS. A solution of trisodium orthophosphate R containing about 2 g of Na_3PO_4 per litre.

Trisodium orthophosphate R. $Na_3PO_4,12H_2O$.
Description. Colourless crystals or a white, crystalline powder.
Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l) TS and carbon disulfide R.

Tropicamide RS. International Chemical Reference Substance.

Tyrosine R. $C_9H_{11}NO_3$ (SRIP, 1963, p. 212).

Uranyl acetate R. $C_4H_6O_6U,2H_2O$ (SRIP, 1963, p. 213).

Uranyl/zinc acetate TS
Procedure. Dissolve 10 g of uranyl acetate R by heating with 50 ml of water and 5 ml of acetic acid (~300 g/l) TS; dissolve 30 g of zinc acetate R by heating with 30 ml of water and 3 ml of acetic acid (~300 g/l) TS. Mix the two solutions, allow to cool to room temperature, and remove by filtration any solid material that separates.

Urea R. CH_4N_2O (SRIP, 1963, p. 214).

Uridine R. 1-β-D-Ribofuranosyluracil; $C_9H_{12}N_2O_6$.
Solubility. Soluble in water.
Melting temperature. About 165 °C.
Storage. Store in a cool place.

Valproic acid RS. International Chemical Reference Substance.

Vanadium pentoxide R. V_2O_5.
Description. A yellow-brown to rust-brown powder.

Solubility. Slightly soluble in water; soluble in concentrated acids and alkalis; practically insoluble in ethanol (~750 g/l) TS.

Vanadium/sulfuric acid TS
Procedure. Dissolve 0.20 g of vanadium pentoxide R in 4 ml of sulfuric acid (~1760 g/l) TS and dilute carefully with water to 100 ml.

Vanillin (10 g/l) TS. A solution of vanillin R containing about 10 g of $C_8H_8O_3$ per litre.

Vanillin R. $C_8H_8O_3$ (SRIP, 1963, p. 214).

Vanillin/hydrochloric acid TS
Procedure. Dissolve 1.0 g of vanillin R in sufficient hydrochloric acid (~250 g/l) TS to produce 100 ml.
Note: Vanillin/hydrochloric acid TS must be freshly prepared.

Vanillin/sulfuric acid TS1
Procedure. Dissolve 5 g of vanillin R in 100 ml of sulfuric acid (~1760 g/l) TS.
Note: Vanillin/sulfuric acid TS1 should be freshly prepared.

Vanillin/sulfuric acid TS2
Procedure. Dissolve 1 g of vanillin R in sufficient ethanol (~750 g/l) TS to produce 100 ml. Carefully add, drop by drop, 2 ml of sulfuric acid (~1760 g/l) TS.
Note: Vanillin/sulfuric acid TS2 must be used within 48 hours.

Verapamil hydrochloride RS. International Chemical Reference Substance.

Vinblastine sulfate RS. International Chemical Reference Substance.

Vincristine sulfate RS. International Chemical Reference Substance.

Warfarin RS. International Chemical Reference Substance.

Water R. Purified water as defined in the monograph for Purified water.
Note: Unless otherwise specified, all solutions indicated in the tests and assays of *The International Pharmacopoeia* are prepared with water R.

Water, ammonia-free, R. Water that complies with the following additional test: to 50 ml add 2 ml of alkaline potassio-mercuric iodide TS; no colour is produced.

Water, carbon-dioxide-free and ammonia-free, R. Ammonia-free water R that has been treated as described under carbon-dioxide-free water R.

Water, carbon-dioxide-free, R. Water that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

Water for injections R. Water for injections as described in the monograph for "Water for injections".

Water BET. Water is suitable if it gives a negative result under the conditions prescribed in 3.4 Test for bacterial endotoxins. It may be prepared by distilling water three times in an apparatus fitted with an effective device to prevent the entrainment of droplets, or by other means which give water of the requisite quality.

Water, sterile, R. Sterile water R that complies with the following additional test:

Pyrogens. Carry out the test as described under 3.5 Test for pyrogens injecting, per kg of the rabbit's weight, 10 ml of water that has been rendered isotonic by the addition of pyrogen-free sodium chloride R.

Water vapour detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for the magnesium perchlorate indicator. The minimum value indicated is 60 µl/l or less, with a relative standard deviation of at most ±20%.

Xanthydrol R. $C_{13}H_{10}O_2$ (SRIP, 1963, p. 210).

Xanthydrol TS

Procedure. Dissolve 20 mg of xanthydrol R in 1 ml of hydrochloric acid (~420 g/l) TS and 99 ml of acetic acid (~300 g/l) TS.

Xylene R. C_8H_{10} (SRIP, 1963, p. 215).

Xylenol orange indicator mixture R

Procedure. Mix 0.1 g of xylenol orange R with 10 g of potassium nitrate R.

Xylenol orange R. [3*H*-2,1-Benzoxathiol-3-ylidene bis[(6-hydroxy-5-methyl-*m*-phenylene) methylenenitrilo]] tetraacetic acid, *S, S*-dioxide, $C_{31}H_{32}N_2O_{13}S$.

Description. An orange powder.

Solubility. Soluble in water and ethanol (~750 g/l) TS.

Yeast extract, water-soluble, R. (SRIP, 1963, p. 215).

Yellow stock standard TS

Procedure. To 9.5 ml of cobalt colour TS, add 1.9 ml of copper colour TS, 10.7 ml of dichromate colour TS, 4.0 ml of iron colour TS, dilute to 100.0 ml with sulfuric acid (~10 g/l) TS, and mix.

Zinc acetate R. $C_4H_6O_4Zn,2H_2O$ (SRIP, 1963, p. 216).

Zinc AsR, granulated. Granulated zinc R that complies with the following tests:

Limit of arsenic. Add 10 ml of stannated hydrochloric acid (~250 g/l) AsTS to 50 ml of water, and apply the general test for arsenic; use 10 g of granulated zinc R and allow the reaction to continue for 1 hour; no visible stain is produced.

Test for sensitivity. Repeat the test for arsenic with the addition of 0.1 ml of dilute arsenic AsTS; a faint, but distinct yellow-coloured stain is produced.

Zinc bis(dibenzyldithiocarbamate) R. $Zn(C_5H_{10}NS_2)_2$.

Description. A white, crystalline powder.

Solubility. Soluble in chloroform R.

Melting range. 178–180 °C.

Zinc bis(dibenzyldithiocarbamate) TS

Procedure. Dissolve 10.0 mg of zinc bis(dibenzyldithiocarbamate) R in sufficient carbon tetrachloride R to produce 100 ml.

Zinc chloride R. $ZnCl_2$ (SRIP, 1963, p. 217).

Zinc R. Zn (SRIP, 1963, p. 216); granulated, powder, or dust.

Zinc standard (20 µg/ml Zn) TS

Procedure. To 4.398 g of zinc sulfate R add 1 ml of acetic acid (~300 g/l) TS and dilute with sufficient water to produce 1000 ml. Dilute 1 ml of this solution to 100 ml with water.

Zinc sulfate R. $ZnSO_4$, $7H_2O$. Contains not less than 99.0% and not more than 105.0% of $ZnSO_4,7H_2O$.

Description. Colourless crystals or a white, crystalline powder; odourless; efflorescent.

Solubility. Very soluble in water; practically insoluble in ethanol (~750 g/l) TS.

Clarity and colour of solution. A 0.05 g/ml solution is clear and colourless.

Chlorides. Dissolve 0.7 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under 2.2.1 Limit test for chlorides; not more than 0.35 mg/g.

Iron. Use 0.4 g; the solution complies with the 2.2.4 Limit test for iron; not more than 0.10 mg/g.

pH value. pH of a 0.05 g/ml solution, 4.4–5.6.

Assay. Dissolve about 0.2 g, accurately weighed, in 5 ml of acetic acid (~60 g/l) TS, and proceed with the titration as described under 2.5 Complexometric titrations. Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 14.38 mg of $ZnSO_4,7H_2O$.

Storage. Store at a temperature below 35 °C, in a tightly closed container.

Zirconyl nitrate R. Contains not less than 43.5% and not more than 45.5% of ZrO_2.

Description. A white powder.

Solubility. Soluble in water giving a solution that is clear or not more than faintly turbid.

Assay. Dissolve about 0.1 g, accurately weighed, in 5 ml of sulfuric acid (~1760 g/l) TS and add carefully 50 ml of water. Add, with stirring, 5 ml of hydrogen peroxide (~330 g/l) TS and 350 ml of diammonium hydrogen phosphate (100 g/l) TS. Add 40 ml of sulfuric acid (~1760 g/l) TS and keep the mixture at a temperature of 40–50 °C for 2 hours. Filter and wash with not more than 200 ml of cold ammonium nitrate (50 g/l) TS until the washings no longer give the reaction A for orthophosphates described under 2.1 General identification tests. Dry and ignite to constant weight. Each g of residue is equivalent to 0.4647 g of ZrO_2.

Zirconyl nitrate TS

Procedure. Dissolve 0.1 g of zirconyl nitrate R in a mixture of 60 ml of hydrochloric acid (~420 g/l) TS and 40 ml of water.

Zuclomifene RS. Clomifene citrate *Z*-isomer. International Chemical Reference Substance.

Supplementary information

Supplementary Information: Contents

International Nonproprietary Names (INN) and graphic formulae

International Nonproprietary Names identify pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is globally recognized and is public property. A nonproprietary name is also known as a generic name.

Guidance concerning the use and selection of INN and the graphic representation of chemical formulae can be found in the following references:

1. Procedure for the selection of recommended international nonproprietary names for pharmaceutical substances and General principles for guidance in devising international nonproprietary names for pharmaceutical substances. The text adopted by the WHO Executive Board in December 2004 is available on the WHO Medicines website (http://www.who.int/medicines/) under "International Nonproprietary Names: Revised Procedure (EB115/11 – 2004)".

2. *International nonproprietary names (INN) for pharmaceutical substances. Cumulative list No. 11.* Geneva, World Health Organization, 2004, available on CD-ROM.

   The INN system as it exists today was initiated in 1950 by a World Health Assembly resolution WHA3.11. The procedure for the selection of recommended international nonproprietary names for pharmaceutical substances, and the general principles for selecting international nonproprietary names for pharmaceutical substances, have been updated regularly since the INN programme began in 1950.

3. Guidelines for the graphic representation of chemical formulae. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report.* Geneva, World Health Organization, 1996, Annex 1 (WHO Technical Report Series, No. 863, pp. 16–49).

   These unique guidelines are intended to help scientists to portray chemical names and structures correctly and unambiguously in pharmacopoeias and other compendia. For details of chemical nomenclature conventions, readers are referred to the recommendations of the International Union of Pure and Applied Chemistry.[1]

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[1] International Union of Pure and Applied Chemistry, Organic Chemistry Division, Commission on the Nomenclature of Organic Chemistry. *Nomenclature of organic chemistry, sections, A, B, C, D, E, F, and H,* 4th ed. Oxford, Pergamon, 1979.

Leigh GJ, ed. *Nomenclature of inorganic chemistry: recommendations 1990.* Oxford, Blackwell Scientific, 1990.

The guidelines should be followed as closely as possible, although it should be noted that unwavering adherence to these principles is not always practicable. Therefore, they may be adapted, with certain exceptions, where necessary to produce accurately drawn structural formulae. Details of the formulae, such as bond lengths, the position of subscripts and superscripts, and the closeness of apposition of the individual atomic symbols, will depend on the drawing method used, whether computer-based or manual.

The guidelines cover acyclic, cyclic, and ionic structures, isotopically modified and coordination compounds, stereochemistry, carbohydrates, steroids, terpenoids, prostanoids, alkaloids, antibiotics, polypeptides, and polymers.

List of International Chemical Reference Substances[1]

International Chemical Reference Substances (ICRS) are established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. They are supplied primarily for use in physical and chemical tests and assays described in the specifications for quality control of drugs published in *The International Pharmacopoeia* or proposed in draft monographs. The ICRS are mainly intended to be used as primary standards to calibrate secondary standards.

Directions for use, and analytical data required for the use described in the relevant specifications of *The International Pharmacopoeia*, are given in the certificates enclosed with the substances when distributed. More detailed analytical reports on the substances may be obtained from the WHO Collaborating Centre for Chemical Reference Substances.

ICRS may also be used in tests and assays not described in *The International Pharmacopoeia*. However, the responsibility for assessing the suitability of the substances then rests with the user or with the pharmacopoeia commission or other authority that has prescribed this use.

It is generally recommended that the substances should be stored protected from light and moisture and preferably at a temperature of about 5 °C. When special storage conditions are required, this is stated on the label or in the accompanying leaflet. It is recommended that the user purchase only an amount sufficient for immediate use.

The stability of the ICRS kept at the Collaborating Centre is monitored by regular re-examination, and any material that has deteriorated is replaced by new batches as necessary. Lists giving control numbers for the current batches are issued in the annual reports from the Centre and new yearly lists may be obtained on request.

Orders for the ICRS should be sent to:

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Sweden
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[1] As updated at the thirty-ninth meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, 24–28 October 2005. Consult the WHO Medicines website (http://www.who.int/medicines/) for the current list.

The International Chemical Reference Substances (ICRS) are only supplied in standard packages as indicated in the following list.

Catalogue number	Reference substances	Package size	Control number
9930375	p-Acetamidobenzalazine	25 mg	290042
9930202	Acetazolamide	100 mg	186128
9930204	Allopurinol	100 mg	287049
9930206	Amidotrizoic acid	100 mg	196205
9930191	2-Amino-5-nitrothiazole	25 mg	186131
9930194	3-Aminopyrazole-4-carboxamide hemisulfate	100 mg	172050
9930193	3-Amino-2,4,6-triiodobenzoic acid	100 mg	196206
9930208	Amitriptyline hydrochloride	100 mg	181101
9930209	Amodiaquine hydrochloride	200 mg	192160
9930210	Amphotericin B	400 mg	191153
9930211	Ampicillin (anhydrous)	200 mg	390001
9930212	Ampicillin sodium	200 mg	388002
9930213	Ampicillin trihydrate	200 mg	274003
9930214	Anhydrotetracycline hydrochloride	25 mg	180096
9931408	Artemether	100 mg	103225
9931406	Artemisinin	100 mg	103222
9931407	Artemotil	100 mg	103226
9931410	Artenimol	100 mg	103223
9931409	Artesunate	100 mg	103224
9930215	Atropine sulfate	100 mg	183111
9930216	Azathioprine	100 mg	172060
9930218	Bacitracin zinc	200 mg	192174
9930219	Beclometasone dipropionate	200 mg	192175
9930225	Benzylpenicillin potassium	200 mg	180099
9930226	Benzylpenicillin sodium	200 mg	280047
9930227	Bephenium hydroxynaphthoate	100 mg	183112
9930228	Betamethasone	100 mg	183113
9930229	Betamethasone sodium phosphate	100 mg	196203
9930230	Betamethasone valerate	100 mg	190145
9930233	Bupivacaine hydrochloride	100 mg	289054
9930234	Caffeine	100 mg	181102
9930236	Calcium folinate (Leucovorin calcium)	100 mg	194188
9930237	Captopril	100 mg	197214
9930238	Captopril disulfide	25 mg	198216
9930239	Carbamazepine	100 mg	189143
9930240	Carbenicillin monosodium	200 mg	383043
9930241	Chloramphenicol	200 mg	486004

Catalogue number	Reference substances	Package size	Control number
9930242	Chloramphenicol palmitate	1 g	286072
9930243	Chloramphenicol palmitate (Polymorph A)	200 mg	175073
9930199	5-Chloro-2-methylaminobenzophenone	100 mg	172061
9930245	Chloroquine sulfate	200 mg	195201
9930190	2-(4-Chloro-3-sulfamoylbenzoyl)benzoic acid	50 mg	181106
9930246	Chlorphenamine hydrogen maleate	100 mg	182109
9930247	Chlorpromazine hydrochloride	100 mg	178080
9930248	Chlortalidone	100 mg	183114
9930249	Chlortetracycline hydrochloride	200 mg	187138
9930250	Cimetidine	100 mg	190150
9930256	Ciprofloxacin hydrochloride	400 mg	197210
9930252	Ciprofloxacin by-compound A	20 mg	198220
9930253	Ciprofloxacin desfluoro-compound	20 mg	198219
9930254	Ciprofloxacin ethylenediamine-compound	20 mg	198218
9930255	Ciprofloxacin fluoroquinolonic acid	20 mg	198217
9930258	Cisplatin	100 mg	197207
9930259	Clomifene citrate	100 mg	187136
	Clomifene citrate Z-isomer *see* Zuclomifene		
9930261	Cloxacillin sodium	200 mg	274005
9930262	Colecalciferol (Vitamin D3)	500 mg	190146
9930263	Cortisone acetate	100 mg	167006
9930265	Dapsone	100 mg	183115
9930266	Desoxycortone acetate	100 mg	167007
9930267	Dexamethasone	100 mg	388008
9930268	Dexamethasone acetate	100 mg	288009
9930269	Dexamethasone phosphoric acid	100 mg	192161
9930270	Dexamethasone sodium phosphate	100 mg	192158
9930282	Diazoxide	100 mg	181103
9930283	Dicloxacillin sodium	200 mg	174071
9930285	Dicoumarol	100 mg	178077
9931413	Didanosine	10 mg	104228
9931414	Didanosine for System Suitability	10 mg	104230
9930287	Diethylcarbamazine dihydrogen citrate	100 mg	181100
9930288	Digitoxin	100 mg	277010
9930289	Digoxin	100 mg	587011
9930290	Dopamine hydrochloride	100 mg	192159
9930292	Doxorubicin hydrochloride	100 mg	196202
9931411	Efavirenz	100 mg	104229
9930294	Emetine hydrochloride	100 mg	187134

Catalogue number	Reference substances	Package size	Control number
9930197	4-Epianhydrotetracycline hydrochloride	25 mg	288097
9930198	4-Epitetracycline hydrochloride	25 mg	293098
9930295	Ergocalciferol (Vitamin D2)	500 mg	190147
9930296	Ergometrine hydrogen maleate	50 mg	277012
9930297	Ergotamine tartrate	50 mg	385013
9930298	Erythromycin	250 mg	191154
9930299	Erythromycin B	150 mg	194186
9930300	Erythromycin C	25 mg	194187
9930301	Estradiol benzoate	100 mg	167014
9930302	Estrone	100 mg	279015
9930304	Ethambutol hydrochloride	100 mg	179081
9930305	Ethinylestradiol	100 mg	301016
9930306	Ethisterone	100 mg	167017
9930307	Ethosuximide	100 mg	179088
9930309	Flucloxacillin sodium	200 mg	195194
9930310	Flucytosine	100 mg	184121
9930311	Fludrocortisone acetate	200 mg	195199
9930312	Fluorouracil	100 mg	184122
9930313	Fluphenazine decanoate dihydrochloride	100 mg	182107
9930314	Fluphenazine enantate dihydrochloride	100 mg	182108
9930315	Fluphenazine hydrochloride	100 mg	176076
9930316	Folic acid	100 mg	388019
9930195	3-Formylrifamycin	200 mg	202149
9930355	Framycetin sulfate (Neomycin B sulfate)	200 mg	193178
9930318	Furosemide	100 mg	171044
9930319	Gentamicin sulfate	100 mg	194183
9930322	Griseofulvin	200 mg	280040
9930323	Haloperidol	100 mg	172063
9930324	Hydrochlorothiazide	100 mg	179087
9930325	Hydrocortisone	100 mg	283020
9930326	Hydrocortisone acetate	100 mg	280021
9930327	Hydrocortisone sodium succinate	200 mg	194184
9930188	(–)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine (3-*o*-Methylcarbidopa)	25 mg	193180
9930189	(–)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine (3-*o*-Methylmethyldopa)	25 mg	179085

Catalogue number	Reference substances	Package size	Control number
9930328	Ibuprofen	100 mg	183117
9930329	Imipramine hydrochloride	100 mg	172064
9930330	Indometacin	100 mg	178078
9930331	Isoniazid	100 mg	185124
9930332	Kanamycin monosulfate	12 mg	197211
9930333	Lanatoside C	100 mg	281022
9930334	Levodopa	100 mg	295065
9930335	Levonorgestrel	200 mg	194182
9930336	Levothyroxine sodium	100 mg	189144
9930337	Lidocaine	100 mg	181104
9930338	Lidocaine hydrochloride	100 mg	181105
9930339	Liothyronine sodium	50 mg	193179
9930340	Loperamide hydrochloride	100 mg	194185
9930341	Mebendazole	200 mg	195195
	Melting Point Reference Substances		
9930217	Azobenzene (69 °C)	1 g	192168
9930438	Vanillin (83 °C)	1 g	299169
9930222	Benzil (96 °C)	4 g	294170
9930201	Acetanilide (116 °C)	1 g	297171
9930380	Phenacetin (136 °C)	1 g	297172
9930221	Benzanilide (165 °C)	1 g	192173
9930422	Sulfanilamide (166 °C)	1 g	192162
9930423	Sulfapyridine (193 °C)	4 g	192163
9930286	Dicyanodiamide (210 °C)	1 g	192164
9930411	Saccharin (229 °C)	1 g	192165
9930235	Caffeine (237 °C)	1 g	299166
9930382	Phenolphthalein (263 °C)	1 g	299167
9930345	Methotrexate	100 mg	194193
	3-*o*-Methylcarbidopa *see* (–)-3-(4-Hydroxy-3-methoxyphenyl)-2-hydrazino-2-methylalanine 3-*o*-Methylmethyldopa *see* (–)-3-(4-Hydroxy-3-methoxyphenyl)-2-methylalanine		
9930346	Methyldopa	100 mg	179084
9930347	Methyltestosterone	100 mg	167023
9930348	Meticillin sodium	200 mg	274024
9930350	Metronidazole	100 mg	183118

Catalogue number	Reference substances	Package size	Control number
9930351	Nafcillin sodium	200 mg	272025
9930354	Neamine hydrochloride (Neomycin A hydrochloride)	0.5 mg	193177
	Neomycin B sulphate *see* Framycetin sulfate		
9930356	Neostigmine metilsulfate	100 mg	187135
9931412	Nevirapine	100 mg	104227
9930357	Nicotinamide	100 mg	200090
9930358	Nicotinic acid	100 mg	179091
9930359	Nifurtimox	100 mg	194189
9930360	Niridazole	200 mg	186129
9930361	Niridazole-chlorethylcarboxamide	25 mg	186130
9930366	Norethisterone	100 mg	186132
9930367	Norethisterone acetate	100 mg	185123
9930369	Nystatin	200 mg	300152
9930371	Ouabain	100 mg	283026
9930372	Oxacillin sodium	200 mg	382027
9930373	Oxytetracycline dihydrate	200 mg	189142
9930374	Oxytetracycline hydrochloride	200 mg	189141
9930376	Papaverine hydrochloride	100 mg	185127
9930377	Paracetamol	100 mg	195198
9930378	Paromomycin sulfate	75 mg	195197
9930383	Phenoxymethylpenicillin	200 mg	179082
9930384	Phenoxymethylpenicillin calcium	200 mg	179083
9930385	Phenoxymethylpenicillin potassium	200 mg	176075
9930387	Phenytoin	100 mg	179089
9930388	Piperazine adipate	100 mg	197212
9930389	Piperazine citrate	100 mg	197213
9930390	Praziquantel	100 mg	194191
9930391	Prednisolone	100 mg	389029
9930392	Prednisolone acetate	100 mg	289030
9930393	Prednisolone hemisuccinate	200 mg	195196
9930394	Prednisolone sodium phosphate	200 mg	194190
9930395	Prednisone	100 mg	167031
9930396	Prednisone acetate	100 mg	169032
9930397	Probenecid	100 mg	192156
9930398	Procaine hydrochloride	100 mg	183119
9930399	Procarbazine hydrochloride	100 mg	184120
9930400	Progesterone	100 mg	167033
9930402	Propranolol hydrochloride	100 mg	187139
9930403	Propylthiouracil	100 mg	185126

Catalogue number	Reference substances	Package size	Control number
9930404	Pyrantel embonate (Pyrantel pamoate)	500 mg	192157
9930405	Pyridostigmine bromide	100 mg	182110
9930406	Reserpine	100 mg	186133
9930407	Retinol acetate (solution)	5 caps (*)	898038
9930408	Riboflavin	250 mg	382035
9930409	Rifampicin	300 mg	191151
9930410	Rifampicin quinone	200 mg	202148
9930412	Sodium amidotrizoate	100 mg	198221
9930413	Sodium cromoglicate	100 mg	188140
9930415	Spectinomycin hydrochloride	200 mg	193176
9930416	Streptomycin sulfate	100 mg	197215
9930417	Sulfacetamide	100 mg	196200
9930419	Sulfamethoxazole	100 mg	179092
9930420	Sulfamethoxypyridazine	100 mg	178079
9930421	Sulfanilamide	100 mg	179094
9930424	Sulfasalazine	100 mg	191155
9930425	Tamoxifen citrate	100 mg	196208
9930426	Tamoxifen citrate *E*-isomer	10 mg	196209
9930427	Testosterone enantate	200 mg	194192
9930428	Testosterone propionate	100 mg	167036
9930429	Tetracycline hydrochloride	200 mg	180095
9930430	Thioacetazone	100 mg	171046
9930196	4,4′ – Thiodianiline	50 mg	183116
	Thyroxine sodium *see* Levothyroxine sodium		
9930431	Tolbutamide	100 mg	179086
9930432	Tolnaftate	100 mg	176074
9930433	Toluene-2-sulfonamide	100 mg	196204
9930434	Trimethadione	200 mg	185125
9930435	Trimethoprim	100 mg	179093
9930440	Vincristine sulfate	9.7 mg/vial	193181
	Vitamin A acetate (solution) *see* Retinol acetate (solution)		
9930439	Warfarin	100 mg	168041
9930260	Zuclomifene	50 mg	187137

(*) About 8 mg in 230 mg oil per capsule

List of International Infrared Reference Spectra[1]

International Infrared Reference Spectra are established on the advice of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Full-scale reproductions of spectra produced from authenticated material on a suitable instrument are supplied for use in identification tests described in the specifications for quality control of drugs, published in *The International Pharmacopoeia* or proposed in draft monographs.

Precise instructions for the preparation of spectra are given on the label of each reference spectrum. All International Infrared Reference Spectra are distributed together with a document giving further details on the use of such spectra, entitled "General recommendations for the preparation and use of infrared spectra in pharmaceutical analysis".[2]

Orders for International Infrared Reference Spectra should be sent to:

WHO Collaborating Centre for Chemical Reference Substances
Apoteket AB
Produktion & Laboratorier Centrallaboratoriet, ACL
Prismavägen 2
S-141 75 Kungens Kurva
Sweden
(Fax: +46 8 740 6040; email who.apl@apoteket.se)

The following International Infrared Reference Spectra are currently available from the Centre:

aceclidine salicylate
acetazolamide
allopurinol
amiloride hydrochloride
amitriptyline hydrochloride
ampicillin trihydrate

beclometasone dipropionate
benzylpenicillin potassium
biperiden
biperiden hydrochloride
bupivacaine hydrochloride

caffeine (anhydrous)
calcium folinate
carbidopa
chlorphenamine hydrogen maleate
clofazimine
cloxacillin sodium
colchicine
cytarabine

dexamethasone
dexamethasone acetate, mono-hydrate

[1] As updated at the thirty-ninth meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, 24–28 October 2005. Consult the WHO Medicines website (http://www.who.int/medicines/) for the current list.

[2] *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report.* Geneva, World Health Organization, 1996, Annex 4 (WHO Technical Report Series, No. 863).

dextromethorphan hydrobromide
diazepam
dicolinium iodide
dicoumarol
diethylcarbamazine dihydrogen citrate
diphenoxylate hydrochloride

erythromycin ethylsuccinate
erythromycin stearate
etacrynic acid
ethionamide
ethosuximide

furosemide

gallamine triethiodide
glibenclamide

haloperidol
hydrochlorothiazide

ibuprofen
imipramine hydrochloride
indometacin
isoniazid

lidocaine
lidocaine hydrochloride
lindane

metronidazole
miconazole nitrate

niclosamide
nicotinamide
noscapine

oxamniquine

papaverine hydrochloride
phenobarbital
phenoxymethylpenicillin calcium
phenytoin
primaquine phosphate
propylthiouracil
protionamide
pyrimethamine

salbutamol
salbutamol sulfate
sulfadimidine
sulfadoxine
sulfamethoxazole
sulfamethoxypyridazine

tiabendazole
trihexyphenidyl hydrochloride
trimethoprim

valproic acid
verapamil hydrochloride

General guidelines for the establishment, maintenance, and distribution of chemical reference substances

Introduction

In 1975, the WHO Expert Committee on Specifications for Pharmaceutical Preparations recommended "General guidelines for the establishment, maintenance and distribution of chemical reference substances" (*1*).[1] At that time these general guidelines were aimed at fostering greater collaboration and harmonization among various national and regional authorities responsible for collections of chemical reference substances. This aim is still relevant. The guidelines were initially drawn up for particular use by the WHO Collaborating Centre for Chemical Reference Substances in Sweden, which provides International Chemical Reference Substances (ICRS). These substances are primarily intended for use with pharmacopoeial monographs included in *The International Pharmacopoeia* (*2*).

It became evident that in order to meet particular national or regional pharmacopoeial requirements, it was necessary to establish chemical reference substances external to the WHO Collaborating Centre for Chemical Reference Substances. Another difficulty was to ensure prompt dispatch of the substances. Since the meticulous work of the WHO Collaborating Centre establishing the international collection would have to be duplicated in local or regional laboratories, guidelines were necessary to ensure the integrity of national or regional collections. In order to clarify the need for national and regional collections, the 1975 guidelines were reviewed and modified in 1982 (*3*). In view of refinements in pharmaceutical and analytical methods since then, the present revision was considered essential.

The purpose of having chemical reference substances is to achieve accuracy and reproducibility of the analytical results required by pharmacopoeial testing and pharmaceutical control in general. These substances are normally prepared and issued by the regional/national pharmacopoeial commission or the regional/national quality control laboratory on behalf of the drug regulatory authority. In the context of these guidelines, the general use of a chemical reference substance should be considered an integral part of a compliance-oriented monograph or test procedure used to demonstrate the identity, purity and content of pharmaceutical substances and preparations.

The establishment of chemical reference substances should be based on reports in which the results of analytical testing have been evaluated. These reports should subsequently be approved and adopted by a certifying body, normally the relevant pharmacopoeial committee or the drug regulatory

[1] The term *chemical reference substances*, as used in this text, refers to an authenticated uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with the properties of a product under examination, and which possesses a degree of purity adequate for its intended use.

authority. Such establishment can be on an international, national or regional basis. Each substance is generally established for a specific analytical purpose, defined by the issuing body. Its use for any other purpose becomes the responsibility of the user and a suitable caution is included in the information sheet accompanying a reference substance. The present guidelines are concerned with both primary and secondary chemical reference substances as defined below.

The preparation of a chemical reference substance should comply with the requirements for quality assurance systems, including principles of good manufacturing practices (GMP) and good control laboratory practices (*4–6*).

Adequate training programmes are also required. Both the WHO Collaborating Centre and other laboratories concerned with the evaluation and establishment of chemical reference substances give assistance in training, subject to the availability of resources.

Primary chemical reference substance

A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context, and whose value is accepted without requiring comparison to another chemical substance.

Secondary chemical reference substance

A secondary chemical reference substance is a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance. The extent of characterization and testing of a secondary chemical reference substance may be less than for a primary chemical reference substance. This definition may apply *inter alia* to some substances termed "working standards".

Part A. Primary chemical reference substances

1. Assessment of need for the establishment of chemical reference substances

The production, validation, maintenance and distribution of chemical reference substances is a costly and time-consuming undertaking. It is, therefore, of great importance to determine critically whether a need for a given substance exists. Requests for new chemical reference substances usually arise when a particular approach to developing a specification for a new substance or product has been adopted. Methods may have been proposed in a specification that require the establishment of a chemical reference substance for use as a comparative standard. Therefore, the first matter that should be assessed is whether an alternative, equally satisfactory, procedure could be adopted that does not require a comparative standard.

Analytical procedures currently used in specifications for pharmaceutical substances and products that may require a chemical reference substance are:

(a) infrared (IR) spectrophotometry, whether for identification or quantitative purposes;
(b) quantitative methods based on ultraviolet (UV) absorption spectrophotometry;
(c) quantitative methods based on the development of a colour and the measurement of its intensity, whether by instrumental or visual comparison;
(d) methods based on chromatographic separation for identification or quantitative purposes;
(e) quantitative methods (including automated methods) based on other separation techniques that depend on partition of the substance to be determined between solvent phases, where the precise efficiency of the extraction procedure might depend upon ambient conditions that vary from time to time and from laboratory to laboratory;
(f) quantitative methods, often titrimetric but sometimes gravimetric, that are based on non-stoichiometric relationships;
(g) assay methods based on measurement of optical rotation; and
(h) methods that might require a chemical reference substance consisting of a fixed ratio of known components (for example, *cis/trans* isomers, spiked samples).

2. Obtaining source material

Source material of satisfactory quality can be selected from a batch (lot) of the substance originating from the normal production process, if the purity is acceptable. Further purification techniques may be needed to render the material acceptable for use as a chemical reference substance.

The purity requirements for a chemical reference substance depend upon its intended use. A chemical reference substance proposed for an identification test does not require meticulous purification, since the presence of a small percentage of impurities in the substance often has no noticeable effect on the test.

On the other hand, chemical reference substances that are to be used in assays should possess a high degree of purity. As a guiding principle, a purity of 99.5% or higher is desirable, calculated on the basis of the material in its anhydrous form or free of volatile substances. However, where the selectivity of the analytical procedure for which the chemical reference substance is required is low, such a degree of purity may not be necessary. In making a decision about the suitability of a chemical reference substance, the most important consideration is the influence of the impurity on the attribute measured in the assay when used in a non-specific assay procedure. Impurities with physicochemical characteristics similar to those of the main component will not impair the usefulness of a chemical reference substance, whereas even traces of impurities with significantly different properties may render a substance unsuitable as a chemical reference substance.

When source material to be used as a chemical reference substance is obtained from a supplier, the following should be supplied with the material:

- Certificate of analysis with complete information as to test methods employed, values found and number of replicates used, where applicable, and relevant spectra and/or chromatograms.
- Information on optimal storage conditions required for stability (temperature and humidity considerations).
- Results of any hygroscopicity study and/or statement of the hygroscopicity of the source material.
- Results of any accelerated stability studies.
- Identification of detected impurities (by preference), and/or specific information on the relative response factor as determined in compendial methods concerning the principal component, and/or the percentage mass of the impurity.
- Updated Material Safety Data Sheet outlining any health hazards associated with the material.

For new drug substances, manufacturers should be aware that elaboration of pharmacopoeial monographs will be needed and a batch of the new substance should be set aside to be used if necessary as the chemical reference substance. It is desirable for bodies that issue chemical reference substances to provide each other with a sample of the same batch of material, even if the substance will be employed for different test methods. This will require the exchange of information concerning the establishment process, supplier(s), availability and conditions of supply.

3. Evaluation of chemical reference substances

The suitability of a substance proposed for use as a chemical reference requires careful evaluation by the issuing body. It is necessary to consider all data obtained from testing the material by a wide variety of analytical methods. When taken as a whole, this will ensure that the substance is suitable for its intended use. The extent of the analyses required depends on the purpose(s) for which the chemical reference substance is to be employed, and may involve a number of independent laboratories.

3.1 Use in identification tests

For use in identification tests (infrared spectrophotometry and/or chromatographic methods), a batch of good quality material selected from the normal production process is satisfactory if it is of acceptable purity. Additional purification by the supplier may be necessary. The most important check is the application of the test(s) for which the substance is intended. It is usual for at least one laboratory to apply all the tests described in the relevant monograph.

3.2 Use in purity tests

The characterization of a chemical reference substance used in the determination of a specific impurity is more extensive, especially when used in a limit test. If the technique employed is thin-layer chromatography (TLC) an acceptable minimum purity is recommended (normally at least 90%), but purer material may be required for liquid chromatography (LC) or gas chromatography (GC). If the proposed reference substance is being prepared or isolated for the first time, appropriate chemical and physicochemical tests, such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and elemental analysis, must be applied to characterize it.

3.3 Use in assays

If the chemical reference substance is to be used in an assay (colorimetry, LC, GC or UV spectrophotometry), the extent of testing is very much greater. Several (a minimum of three) laboratories should collaborate in testing the proposed substance, using a variety of established and validated techniques, including the method used in the pharmacopoeial specification. The relative reactivity or relative absorbance of the impurities present must be checked when a non-specific assay method is employed, e.g. by colorimetry or UV spectrophotometry. When a selective assay method is employed, it is particularly important to determine the quantity of impurities. In such a case, it is best to examine the proposed reference substance by as many methods as practicable including, where possible, absolute methods. For substances that are acidic or basic a titration with alkali or acid is simple, but other reactions which are known to be stoichiometric may be used. Phase solubility analysis and differential scanning calorimetry may also be employed in certain cases.

The total of the determinations of water content, organic solvents, mineral impurities and organic components should amount to 100%. For most chemical reference substances intended for assays, the content may be expressed "as is". When establishing the chemical reference substance it is, therefore, essential to determine the content of water and residual solvents for a non-specific assay, and also to determine the content of impurities for a selective assay.

3.4 Use in the calibration of an instrument

Where the chemical reference substance is to be employed as calibration material, the extent of testing is similar to that for a chemical reference substance used in assays. Several laboratories should collaborate in testing the proposed substance using a variety of techniques to check that its purity is adequate. An appropriate number of collaborating laboratories should also participate, after the reference substance has been deemed suitable, to establish a value for the essential property of the substance using an appropriate instrument.

4. Chemical and physical methods used in evaluating chemical reference substances

It is important to establish by individual testing that a substance proposed for use as a chemical reference is suitable.

The methods used to establish the suitability of such a substance fall into two broad groups: those intended primarily to identify the substance and those used to establish its purity. With most methods, the percentage purity of a chemical reference substance cannot be expressed as an absolute value if the impurities have not been identified. The quoted purity is then an estimate based upon the data obtained by the various analytical methods.

4.1 Methods used to verify the identity of chemical reference substances

Where a proposed substance consists of a compound whose structure has been satisfactorily defined, its identity may be confirmed by matching the IR spectra of the substance to that of an authentic compound. Particular care should be taken when polymorphism exists (7). Other highly specific techniques, such as NMR spectroscopy, MS, or X-ray diffraction crystallography, may also be used for such comparisons. The identity of a substance that is intended to replace an established chemical reference substance of the same molecular constitution must be verified, to determine that the characteristic properties of the two specimens are identical. For this purpose it is often sufficient to compare their IR absorption spectra.

However, where no authentic specimen of the proposed substance is available for comparison, and definitive data about its properties are lacking, it may be necessary to verify its identity by applying several analytical techniques currently used to characterize new compounds. Such analytical methods may include elemental analyses, crystallographic studies, MS, NMR spectroscopy, functional group analyses, and IR or UV spectrophotometry, as well as other supplementary tests as required to establish that the proposed substance is fully characterized.

4.2 Methods used to determine the purity of chemical reference substances

The analytical methods to be employed in examining a substance should be considered in relation to its intended use. These analytical methods may be divided into three broad categories: those that require comparison with an external chemical reference substance (e.g. chromatographic or spectrophotometric methods), those that depend solely on an intrinsic dynamic property (e.g. phase solubility analysis and differential scanning calorimetry) and other methods.

4.2.1 *Separation techniques*

The methods used for the determination of purity should be established and validated with system suitability requirements as appropriate.

Chromatographic methods. Methods of analysis based on chromatographic separation are especially useful for detecting and determining impurities in chemical reference substances. High-performance liquid chromatography (HPLC) is the most widely used chromatographic method, but TLC and GC are also used. The individual components separated by chromatographic methods may sometimes be recovered for characterization.

The selectivity of HPLC and of GC usually exceeds that of TLC. Both the first two methods also have the advantage of being readily applicable on a quantitative basis, but they require more complex equipment. HPLC, employing a spectrophotometric method of detection, is of particular value in the examination of chemical reference substances intended for use in UV spectrophotometric assays. The UV wavelength of detection employed for determining the impurity content of the chemical reference substance should be chosen so that the detection responses of the substance and its known impurities are similar. When the response factors are significantly different at the optimal wavelength of detection, appropriate corrections must be made to estimate the content of impurities. LC with diode-array detection is very useful for recording the UV spectra of both the main peak and the impurities. LC with MS detection is used for identification of separated impurities as well as for the main component, and is particularly important for chemical reference substances where no other reference standards or IR reference spectra are available.

In a GC method used for an assay, as with LC, the detection responses of the known impurities are determined. Generally, GC monograph methods are of particular value in detecting and determining volatile impurities, including solvent residues, in chemical reference substances.

TLC uses apparatus that is simple and cheap; the technique is easy to carry out and is readily applicable even in the microgram range. It can separate closely related compounds, such as geometric isomers and the members of a homologous series. All the constituents of a substance submitted to chromatography appear somewhere on the chromatogram. However, some constituents may remain on the starting line, some may move with the solvent front, some may migrate at the same rate as the main component, and some may remain undetected. For this reason, the usefulness of the method may be greatly enhanced by means of two-dimensional chromatography and by using a number of different solvent systems and a variety of detection methods. In some cases the method may be used quantitatively with acceptable accuracy by using a densitometer.

Capillary electrophoresis. Capillary electrophoresis is an increasingly common method. It may be considered as complementary to LC for detecting impurities.

4.2.2 *Methods based on intrinsic thermodynamic properties*

Methods in this group measure total impurity levels in absolute terms.

Differential scanning calorimetry. This technique is used to check the presence of different polymorphic forms and to determine the total amount of solid impurities. Purity estimation is based on determination of the heat of fusion of the sample and of the change in its melting point caused by the presence of impurities. This analytical method can be performed rapidly and with high precision. However, it is not applicable if the substance decomposes on melting. This limits its value as a general procedure for purity estimation of chemical reference substances. It is also inapplicable if solid solutions are formed.

Phase solubility analysis. The method has occasionally been used, but its value is limited and the procedure is time consuming. It may be employed to detect contaminating substances, including isomeric species, and to estimate their concentration. Some factors that may make the method inapplicable are degradation of the substance during the course of analysis, formation of a solid solution, and polymorphism in the main component.

4.2.3 *Other methods*

Spectrophotometric methods. UV spectrophotometry is occasionally used to determine purity. Since it depends upon the presence of a characteristic chromophore, it can detect impurities that contribute excessively to the absorbance value and may indicate the presence of impurities that have a negligible or distinctive absorbance.

However, the utility of the method is limited by the small number of absorption maxima in the UV range, the large numbers of compounds containing similar characteristic chromophores, and the need for an external chemical reference substance.

IR spectrophotometry may be used to identify and determine the proportions of geometric isomers. NMR spectroscopy, a powerful spectroscopic identification tool, is also occasionally useful in the determination of purity.

Titrimetric methods. Titrimetric methods provide a valuable means of confirming the identity and purity of a proposed chemical reference substance and are useful in confirming purity values obtained by other methods.

Optical rotation methods. Many chemical reference substances are optically active and the relative proportion of optical isomers can sometimes be determined by an optical rotation method, but generally such methods lack sensitivity. However, the quantitative use of these techniques is well established and can yield results of high precision, depending on the solvent and the wavelength chosen for measurement. Chiral chromatography and NMR are becoming increasingly important.

Determination of water and organic volatiles. It is essential that an accurate assessment of the moisture content and the content of volatile contaminants be made. These total values may often be obtained by drying under defined conditions that are appropriate to the proposed substance. Sometimes this may not be

possible or may yield misleading results. In such cases, thermogravimetric analysis may be used to determine the water and volatile content. Alternatively, the water content may be determined by Karl Fischer titration and the content of volatile solvents by GC. Without an accurate assessment of these values at the time that other determinations are being made, judgements of the acceptability of the proposed chemical reference substance will be invalid.

5. Assignment of content

If a content is to be assigned to a chemical reference substance, it should be borne in mind that the value is based on the results of a collaborative interlaboratory programme using different analytical methods. This experimentally obtained value represents the best estimate of the true value. In general, the assignment of content for a chemical reference substance is 100% minus the content of water and volatiles, and when a substance is intended for use as an assay standard based on a separation technique the impurity content, as determined by that method, must also be subtracted. Sometimes the chemical reference substances must be dried before use, in which case the content is expressed on the basis of the dried material.

6. Handling and distribution of chemical reference substances

The handling, distribution and use of established chemical reference substances must ensure that their integrity is safeguarded and maintained throughout their period of use.

6.1 Packaging operations

Current GMP requirements (*5*) should be observed. The various stages in packaging chemical reference substances should be clearly defined and controlled, to avoid contamination of the sample, mislabelling of containers, or any other event which might result in mishandling or mismanagement.

Containers for chemical reference substances should protect their contents from moisture, light and oxygen and must be tested for moisture permeability.

Additional measures may be necessary to ensure long-term integrity and stability. The best containers for chemical reference substances from the point of view of stability are sealed glass ampoules, but these have certain disadvantages. There is the risk of contaminating the substance with glass particles when the ampoules are opened, and reclosure is difficult. Sealable glass ampoules are therefore principally used for substances that must be kept in an oxygen-free atmosphere. Certain other substances may require even more elaborate protection. Most chemical reference substances, however, are conveniently supplied in reclosable containers which should be uniform in type and size to facilitate distribution. The lack of permeability to moisture is an important factor in determining the suitability of container closure systems.

Before undertaking any packaging operations, the health hazards of the item to be packaged should be assessed through information sources, e.g. the

Material Safety Data Sheet. Appropriate precautions should be taken to protect the person handling the chemical reference substance.

The packaging of a batch of a chemical reference substance into containers is a small-scale operation for which suitable equipment is now always available to the manufacturer of the material. Therefore, the packaging of chemical reference substances is usually undertaken by the responsible issuing body. Screw-type feeders have been constructed, but generally the packaging of chemical reference substances is carried out manually. Substances which are expensive or only available in very small quantities may have to be divided between containers in solution and then lyophilized, or evaporated to dryness.

Some chemical reference substances must be packaged under an inert gas or in conditions of controlled humidity. Therefore, the use of a glove-box or an airtight cabinet is necessary.

6.2 Storage

Information about suitable storage conditions can often be obtained from the manufacturer of the source material and should be requested routinely when a new chemical reference substance is established. Theoretically, the stability of the substances should be enhanced by keeping them at low temperatures but, for substances that contain water, storage below 0 °C may impair the stability. It should also be remembered that the relative humidity in normal refrigerators or cold-rooms may be high and, unless ampoules or other tightly closed containers are used, the improvement in stability may be more than offset by degradation due to the absorption of moisture. Storage at about +5 °C, with precautions to prevent such absorption, has proved satisfactory for most chemical reference substances.

6.3 Stability

A chemical reference substance is an integral part of the drug specification. Thus, if the reference substance deteriorates, this will change the specification of the drug. It is, therefore, of the utmost importance that the stability of chemical reference substances should be monitored by regular re-examination and that they should be replaced as soon as a significant change in a property is noted.

The definition of what is a "significant change" differs according to the intended use of the chemical reference substance. Several per cent of degradation products found in a substance may not impair the usefulness of the material in identification tests. For chemical reference substances that are used in chromatographic assays, however, even small amounts of impurities may be unacceptable. When establishing a chemical reference substance, consideration must be given to its intended use and to the performance characteristics of the analytical methods in which it will be used. The tolerable degree of degradation will be different from case to case.

Laboratories in charge of collections of chemical reference substances should have a system for regular re-examination of the materials in stock. The frequency of re-testing may be modified according to the need. It must be borne in mind that the stability of a specially prepared chemical reference substance may not always be the same as that of commercial samples of the same material.

The selection of suitable analytical methods for monitoring the stability of chemical reference substances depends on the nature and intended use of the substance. A substance used solely for identification purposes will normally only require demonstration that it is still suitable for this use, e.g. that the IR spectrum is identical to that obtained during establishment. If substances are employed for other purposes, the testing must be more extensive but should use methods which are rapid and sensitive so as not to consume too much of the existing stock. It is important to check that there has been no significant uptake of moisture, which could result in degradation by hydrolysis and/or a decrease in the assigned content of the substance. Chromatography is employed extensively, as well as absolute methods such as differential scanning calorimetry where applicable. Changes in the impurity profile or purity determination usually mean that the batch must be replaced. Changes which compromise the integrity of the batch indicate it should immediately be withdrawn from use. Sometimes a batch of a chemical reference substance will discolour or otherwise change in appearance. Steps should be taken to replace this substance whether or not the results of subsequent analyses indicate significant degradation. Such changes in physical appearance reduce the confidence of the user in the suitability of the chemical reference substance. Appropriate testing of active bulk substance should be carried out before further dispensing into vials or ampoules.

6.4 Information to be supplied with chemical reference substances

Labels on chemical reference substances should give the following information:

— the appropriate name of the substance: the international nonproprietary name (INN) should be used wherever possible;
— name and address of the issuing body;
— approximate quantity of material in the container; and
— batch or control number.

Where associated documents are provided they should incorporate relevant items from the list above. The following information should be given, as necessary, on the labels and/or in associated documents:

— recommended storage conditions (if special conditions apply);
— intended use of the chemical reference substance;
— directions for use (e.g. storage and handling);

— information about assigned analytical value of the chemical reference substance (needed for calculation of the results of tests in which the substance will be used);
— a disclaimer of responsibility when chemical reference substances are misused, or stored under inappropriate conditions, or used for other purposes than those intended by the issuing body; and
— health hazard information or warning in conformity with national and regional regulations or international agreements.

If analytical data are to be supplied with the chemical reference substances, it is recommended that the data provided be limited to what is necessary for the proper use of the substances in the tests and assays.

6.5 Distribution and supply

Distribution of chemical reference substances within the same country usually does not present problems. However, when samples are to be sent to other countries, both the sender and the receiver of the goods may encounter difficulties because of the vagaries of postal and customs regulations, e.g. the application of special procedural requirements applicable to substances under international control. Distributors of chemical reference substances waste considerable resources in seeking information on different international import regulations, and in completing the required forms. A way of reducing such difficulties and barriers to effective distribution of chemical reference substances should be sought. There should be the minimum delay in providing the chemical reference substances to the users, and the most speedy means of transport should be chosen.

6.6 Period of use

Chemical reference substances do not carry an "expiry date" in the conventional sense. To avoid the unnecessary discarding of satisfactory substances, a mechanism for general control of the batch of a chemical reference substance may be used by the issuing body. If the issuing body applies stability considerations and a monitoring procedure based on its experience to its collection, this should guarantee the user of the acceptability of the chemical reference substance for its intended use.

If it is considered necessary to specify a beyond-use date, it should be stated on the label and/or on a document accompanying the chemical reference substances. Adequate shipping records should exist to enable contact with the purchaser of a batch for recall or other notification.

The storage and maintenance of unopened containers of the chemical reference substance in accordance with information provided are integral to its suitability of use. To avoid potential doubts concerning the integrity of opened containers, it is suggested that potential users obtain only the quantities of

substances necessary for short-term need and obtain fresh stocks (held under controlled and known conditions) when needed. Long-term storage of substances in opened containers is to be avoided. Similarly, efforts should be made to avoid possible degradation, contamination and/or introduction of moisture during the repeated use of a substance.

Part B. Secondary chemical reference substances

The establishment of secondary chemical reference substances calibrated against a primary chemical reference substance may be desirable for various practical reasons, e.g. the latter may not be available in adequate quantities to supply all local needs. Moreover, the availability of such secondary chemical reference substances (for example, on a regional basis) would reduce the delay in receiving the reference material.

The body which establishes a secondary chemical reference substance for national/regional use should be clearly defined by the competent drug regulatory authority. Clear documentation must exist to establish the relationship between the secondary and the primary chemical reference substance.

References

The references below are those quoted in the guidelines published as Annex 3 to the Thirty-fifth Report of the Expert Committee on Specifications. Geneva, World Health Organization, 1999 (WHO Technical Report Series, No. 885). For current WHO guidelines on, for example, GMP, consult the WHO Medicines website (http://www.who.int/medicines).

1. *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Twenty-fifth report.* Geneva, World Health Organization, 1975, Annex 3 (WHO Technical Report Series, No. 567).
2. *The International Pharmacopoeia*, 3rd ed. *Vol. 1. General methods of analysis; Vol. 2. Quality specifications; Vol. 3. Quality specifications; Vol. 4. Tests, methods, and general requirements. Quality specifications for pharmaceutical substances, excipients, and dosage forms.* Geneva, World Health Organization, 1979–1994.
3. *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Twenty-eighth report.* Geneva, World Health Organization, 1982, Annex 1 (WHO Technical Report Series, No. 681).
4. Good laboratory practices in governmental drug control laboratories. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirtieth report.* Geneva, World Health Organization, 1987, Annex 1 (WHO Technical Report Series, No. 748).
5. Good manufacturing practices for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 823).
6. Good manufacturing practices for biological products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-third report.* Geneva, World Health Organization, 1993, Annex 3 (WHO Technical Report Series, No. 834).
7. General recommendations for the preparation and use of infrared spectra in pharmaceutical analysis. In: *WHO Expert Committee on Specifications for Pharmaceutical*

Preparations. Thirty-fourth report. Geneva, World Health Organization, 1996, Annex 4 (WHO Technical Report Series, No. 863).

Recommendations on Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This definition applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, e.g. as raw or source materials, starting materials or reagents.

Materials of animal origin should be avoided whenever possible. However, if used, manufacturers should be aware of the risk and have a system in place to minimize it, especially since international trading patterns often include the processing and re-export of products, so that their origin may not be traceable. In order to minimize the risk of transmitting animal spongiform encephalopathy agents via medicinal products, manufacturers should follow the current WHO Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products[1] and the recommendations of the Joint Technical Consultation on Bovine Spongiform Encephalopathy, public health, animal health and trade, convened by the WHO, the Food and Agriculture Organization of the United Nations and the Office International des Epizooties 11–14 June 2001 in Paris.[2]

[1] Note: the current guidelines are published on the WHO website [www.who.int/bloodproducts/tse]. They were adopted by ECBS in 2003; they were reviewed in 2005 and are likely to be updated.

[2] Proceedings – Joint WHO/FAO/OIE Technical Consultation on BSE: public health, animal health and trade. Paris: Office International des Epizooties, World Health Organization, Food and Agriculture Organization, World Organisation for Animal Health, 2002.

Index

Abbreviations used:

AsR	reagent for arsenic limit test
AsTS	test solution for arsenic limit test
BET	bacterial endotoxin test
ClTS	test solution for chloride limit test
Cm	culture medium
FeTS	test solution for iron limit test
IR	reagent for infrared spectrophotometry
PbTS	test solution for lead limit test
R	reagent
RS	International Chemical Reference Substance
TS	test solution
VS	volumetric solution

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Basic tests for drugs:
pharmaceutical substances, medicinal plant materials and dosage forms
1998 (94 pages)

Basic tests for pharmaceutical dosage forms
1991 (134 pages)

Quality Assurance of Pharmaceuticals: a compendium of guidelines and related materials
Volume 1: 1997 (244 pages)
Volume 2: good manufacturing practices and inspection. 2004 (236 pages)

WHO Expert Committee on Specifications for Pharmaceutical Preparations
Fortieth report
WHO Technical Report Series, No. 937,
2006 (461 pages)

International nonproprietary names (INN) for pharmaceutical substances
Cumulative list no. 11
2004 (available in CD-ROM format only)

The use of essential medicines
Report of the WHO Expert Committee
(including the 13th Model List of Essential Medicines)
WHO Technical Report Series, No. 920,
2004 (133 pages)

WHO Expert Committee on Biological Standardization
Fifty-fourth report
WHO Technical Report Series, No. 927,
2005 (160 pages)

Further information on these or other WHO publications can be obtained from WHO Press, World Health Organization, 1211 Geneva 27, Switzerland.
Attention is also drawn to the WHO Medicines web site
(http://www.who.int/medicines)

The International Pharmacopoeia

The International Pharmacopoeia (Ph. Int.) constitutes a collection of recommended procedures for analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms that is intended to serve as source material for reference or adaptation by any WHO Member State wishing to establish pharmaceutical requirements. The pharmacopoeia, or any part of it, shall have legal status, whenever a national or regional authority expressly introduces it into appropriate legislation. Further explanation of the role of *The International Pharmacopoeia* is provided in the paragraphs entitled "Scope and function" at the end of the Preface to this edition.

This is the fourth edition of *The International Pharmacopoeia*. It comprises two volumes; the General Notices and monographs for pharmaceutical substances (A to O) are to be found in Volume 1 and the remaining monographs for pharmaceutical substances together with those for dosage forms and radiopharmaceutical preparations, the methods of analysis and the reagent sections are to be found in Volume 2.

World Health Organization

ISBN 92 4 156401 X