The Ministry of Health, Labour and Welfare Ministerial Notification No. 395

In accordance with the provision of Paragraph 1 of Article 41 of the Pharmaceutical Affairs Law (Low No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 111, 2001) as follows, and the revised Japanese Pharmacopoeia shall come into effect on January 1, 2003. The drugs included in the Japanese Pharmacopoeia (hereinafter referred to as ''the old Japanese Pharmacopoeia'') [limited to those included in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as ''the new Japanese Pharmacopoeia'')] and the approval for which is given on January 1, 2003 as stipulated in Paragraph 1 of Article 14 (including cases where it shall apply *mutatis mutandis* under Article 23 of the Law; the same hereinafter) [including the drugs designated as those exempted from manufacturing or import approval (Ministerial Notification on the designation of the drugs exempted from manufacturing or import approval in accordance with the provision of Paragraph 1 of Article 14 of the Law) (hereinafter referred to as ''the drugs exempted from approval'')] can be treated, up to June 30, 2004, in such a way that the standards established by the old Japanese Pharmacopoeia (limited to the contents concerned with the relevant drugs) may be accepted as those established by the new Japanese Pharmacopoeia. The drugs included in the new Japanese Pharmacopoeia (excluding those in the old Japanese Pharmacopoeia) the approval for which is given on January 1, 2003, as stipulated in Paragraph 1 of Article 14 of the Law (including the drugs exempted from approval), can be treated, up to June 30, 2004, as those not included in the new Japanese Pharmacopoeia.

Chikara Sakaguchi
The Minister of Health, Labour and Welfare

December 27, 2002

(The texts referred to by the term ''as follows'' are omitted here. All of them are made available for public exhibition at the Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labour and Welfare, at each Regional Bureau of Health and Welfare and at each Prefectural Office in Japan.)

The term ''as follows'' here indicates the contents from Part I to Ultraviolet-visible Reference Spectra in the Supplement I to the Japanese Pharmacopoeia Fourteenth Edition (pp.1359 – 1610).

CONTENTS

Preface

The Fourteenth Edition of the Japanese Pharmacopoeia was promulgated on March 30, 2001 by Ministerial Notification No. 111 of the Ministry of Health, Labour and Welfare. To keep pace with progress in medical and pharmaceutical sciences, in November 2001, the Council, at a meeting of the Committee on Japanese Pharmacopoeia (JP), established the basic principles for the preparation of the JP Fifteenth Edition, setting out the characteristics and roles of the JP, the definite measures for the revision, the date of the revision, and the organization of the Subcommittee on JP.

At the above meeting, the following ''five pillars'' were established as the basic principles of the JP: Making it more substantial by including all drugs which are important from the viewpoint of health care and medical treatment; Making prompt partial revision as necessary and facilitating smooth administrative operation; Promoting international harmonization; Ensuring transparency regarding the revision and dissemination to the public of the JP; and Positively introducing contemporary analytical tests and developing reference standards. It was decided at the meeting that each panel set up under the Subcommittee on JP should make efforts, on the basis of these principles, to ensure that the JP is used more effectively in the fields of health care and medical treatment by taking appropriate measures, including getting the understanding and cooperation of other parties concerned.

The JP should comprise an official standard being required to assure the quality of drugs in this country in response to the progress in science and technology and clinical demands at the time, it should define the standards for specifications as well as the methods of tests to assure the overall quality of all drugs in principle, and it should have a role in clarifying the criteria for quality of drugs which are recognized to be important from the viewpoint of medical treatment.

At the same time, it was agreed that the JP should be prepared with the aid of the knowledge and experience of many persons involved in the pharmaceuticals, that it should have the characteristics of an official standard, which might be widely used by all parties concerned, that it should provide information and understanding about the quality of drugs to the public, and that it should be conducive to smooth and effective government control of the quality of drugs, and to securing and maintaining international consistency.

It was also agreed that JP articles should cover drugs which are important from the viewpoint of health care and medical treatment, clinical results and frequency of use, as soon as possible after they reach the market.

It was also decided to make a definite rule for selection of articles, by clarifying the significance of, and standards for selection. The JP Fifteenth Edition was decided to be slated for completion in April 2006.

Under the Subcommittee on JP, the following twelve panels and two provisional panels were established at first: Panel on Planning and Revisions; Panel on the Selection of Articles; Panel on Nomenclature; Panel on Excipients; First Panel on Medicinal Chemicals; Second Panel on Medicinal Chemicals; Panel on Biologically Derived Drugs; Panel on Biological Tests; Panel on Physico-chemical Tests; Panel on Material Sciences; Panel on Preparations; Panel on Crude Drugs; Provisional First Panel on Antibiotics; Provisional First Panel on Crude Drugs. Some of the names of the above panels were changed as follows, due to the reorganization of the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in November 2001: Panel on Nomenclature to Panel on Nomenclature for Pharmaceutical Chemicals; First Panel on Medicinal Chemicals and Second Panel on Medicinal Chemicals were integrated into Panel on Medicinal Chemicals; Panel on Physico-chemical Tests, Panel on Material Sciences and Panel on Preparations were integrated into Panel on Physico-chemical Tests; Panel on Crude Drugs and Provisional First Panel on Crude Drugs were integrated into Panel on Crude Drugs; Provisional First Panel on Antibiotics to Panel on Antibiotics. Under the Panel on Planning and Revisions, the following two panels were newly established: Panel on General Revisions and Panel on Pharmacopoeial Harmonization (PDG).

In the Committee on Japanese Pharmacopoeia, Tadao Terao took the role of chairman from November 1997 to December 2000 and Mitsuru Uchiyama from January 2001 to December 2002.

With the reform of central government ministries and agencies in January 2001, the Ministry of Health and Welfare became the Ministry of Health, Labour and Welfare, and the Committee on Japanese Pharmacopoeia (CJP) came under the authority of the

Minister of Health, Labour and Welfare. At the same time, the Central Pharmaceutical Affairs Council became the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) and Mitsuru Uchiyama was nominated as chairman of the CJP.

It was decided that the JP will be revised not only every five years, in line with the basic principles for the preparation of the JP Fifteenth Edition, but also as necessary to take account of recent progress of science and in the interests of international harmonization. In accordance with the revision principles, the panels continued discussions on selection of articles, and revisions for General Notices, General Rules for Preparations, General Tests, and monographs on drugs.

Among the items discussed, the following two revision drafts were separately examined by the Committee on JP in November 2001, followed by PAFSC in December 2001, and then submitted to the Minister of Health, Labour and Welfare, and Ministerial Notification No.151 promulgated these revisions on March 29, 2002: Addition of a paragraph ''In principle, animals used as a source of materials for preparing pharmaceutical drugs must be healthy.'' to General Notices; and Deletion of a monograph ''Phenacetin''.

Draft revisions covering subjects in General Notices, General Rules for Preparations, General Rules for Crude Drugs, General Tests, and monographs on drugs, for which discussions were finished between June 2000 and February 2001, were prepared for a supplement to the book. They were examined by the Committee on JP in September 2002, followed by PAFSC in December 2002, and then submitted to the Minister of Health, Labour and Welfare.

Numbers of discussions in the panels to prepare supplement drafts were as follows: Panel on the Principles of Revisions, 6 times; Panel on the Selection of Articles, 2 times; Panel on Nomenclature, 11 times; Panel on Excipients, 10 times; First Panel on Medicinal Chemicals, 10 times; Second Panel on Medicinal Chemicals, 16 times; Panel on Biologically Derived Drugs, 8 times; Panel on Biological Tests, 9 times; Panel on Physico-chemical Tests, 8 times; Panel on Material Sciences, 8 times; Panel on Preparations, 5 times; Panel on Crude Drugs, 6 times; Provisional First Panel on Antibiotics, 27 times; Provisional First Panel on Crude Drugs, 7 times. Numbers of discussion in the panels, which were renamed due to the reorganization of PAFSC, were as follows: Panel on Nomenclature for Pharmaceutical Chemicals, 2 times; Panel on Physico-chemical Tests, 1 time; Panel on Crude Drags, 3 times; Panel on General Revisions, 1 time.

It should be noted that in the preparation of the drafts for the Supplement I, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer's Association of Tokyo and of Osaka, the Crude Drugs Association of Tokyo, the Japan Pharmaceutical Excipients Council, the Federation of Crude Drugs Associations of Japan, the Japan Antibiotics Research Association, the Japan Flavor and Fragrance Manufacturer's Association, the Japan Medical Plants Federation, the Japan Pharmaceutical Manufacturer's Association, the Japanese Society of Hospital Pharmacists, the Japan Pharmaceutical Association, and the Japan Oilseed Processors Association.

In consequence of this revision, the JP Fourteenth Edition carries 881 articles in Part I owing to the addition of 31 articles and the deletion of 8 articles; and 481 articles in Part II owing to the addition of 15 articles and the deletion of 3 articles.

The principles of description and the salient points of the revision in this volume are as follows:

1. The Supplement I to JP Fourteenth Edition comprises the following items, in order: Notification of the Ministry of Health, Labour and Welfare; Contents; Preface; followed by General Notices; General Rules for Preparations; General Tests, Processes and Apparatus; Monographs on Drugs in Part I, and General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; Monographs on Drugs in Part II, then followed by Infrared Reference Spectra in Part I; Ultraviolet-visible Reference Spectra in Part I; General Information, and as appendix, the Ministry of Health, Labour and Welfare Ministerial Notification No. 151 (March 2002); and Cumulative Index containing references to the main volume and the supplement I.

2. The articles in General Rules for Preparations, in General Tests, Processes and Apparatus, Monographs on Drugs, Infrared Reference Spectra and Ultraviolet-visible Reference Spectra are respectively placed in alphabetical order.

3. The following items in each monograph are put in the order shown below, except that unnecessary items are omitted depending on the nature of the drug:

(1) English title
(2) Commonly used name(s)
(3) Latin title (only for Crude Drugs)
(4) Title in Japanese
(5) Structural formula or empirical formula
(6) Molecular formula and molecular mass
(7) Chemical name

(8) Origin
(9) Limits of the content of the ingredient(s) and/or the unit of potency
(10) Labeling requirements
(11) Method of preparation
(12) Description
(13) Identification tests
(14) Specific physical and/or chemical values
(15) Purity tests
(16) Loss on drying, loss on ignition, and/or water
(17) Residue on ignition, total ash, and/or acid-insoluble ash
(18) Special tests
(19) Isomer ratio
(20) Assay or the content of the ingredient(s)
(21) Containers and storage
(22) Expiration date
(23) Others

4. In each monograph on a drug, the following physical and chemical values representing the properties and quality of the drug are given in the order indicated below, except that unnecessary items are omitted depending on the nature of the drug:
(1) Alcohol number
(2) Absorbance
(3) Congealing point
(4) Refractive index
(5) Osmolarity
(6) Optical rotation
(7) Viscosity
(8) pH
(9) Specific gravity
(10) Boiling point
(11) Melting point
(12) Acid value
(13) Saponification value
(14) Ester value
(15) Hydroxyl value
(16) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:
(1) Coloration reactions
(2) Precipitation reactions
(3) Decomposition reactions
(4) Derivatives
(5) Visible, ultraviolet or infrared spectra
(6) Special reactions
(7) Cations
(8) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of the drug:
(1) Color
(2) Odor
(3) Clarity and/or color of solution
(4) Acidity or alkalinity
(5) Acid
(6) Alkali
(7) Chloride
(8) Sulfate
(9) Sulfite
(10) Nitrate
(11) Nitrite
(12) Carbonate
(13) Bromide
(14) Iodide
(15) Soluble halide
(16) Thiocyanide
(17) Selenium
(18) Cationic salts
(19) Ammonium
(20) Heavy metals
(21) Iron
(22) Manganese
(23) Chromium
(24) Bismuth
(25) Tin
(26) Aluminum
(27) Zinc
(28) Cadmium
(29) Mercury
(30) Copper
(31) Lead
(32) Silver
(33) Alkaline earth metals
(34) Arsenic
(35) Foreign matter
(36) Related substances
(37) Other mixtures
(38) Readily carbonizable substances

7. Revisions in the General Notices are as follows:
(1) In paragraph 6, an abbreviation ''lx'' for lux was added as the main unit.
(2) In paragraph 8, the definition for ''a cold place'' was revised to as ''a place having a temperature between 1 and 15°C''.

8. Revision in the General Rules for Preparations is as follows:
(1) Injections: The description with respect to the injection having 50 mL or more was deleted from section (9).

9. The following items of the General Tests,

Processes and Apparatus were partially revised:
(1) Infrared Spectrophotometry
(2) Residue on Ignition Test
(3) Vitamin A Assay

10. The following tests were added to the General Tests, Processes and Apparatus:
(1) Conductivity Measurement
(2) Determination of Bulk and Tapped Densities

11. The following Reference Standard was deleted:
Drostanolone Propionate

12. The following Reference Standard was renamed:
Kitasamycin to Leucomycine A_5

13. The following Reference Standards were added:
Aceglutamide
Acetylspiramycin II
Aclarubicin
Actinomycin D
Arbekacin Sulfate
Astromicin Sulfate
Bacitracin
Bekanamycin Sulfate
Benzylpenicillin Potassium
Benzylpenicillin Sodium
Bleomycin A_2 Hydrochloride
Carumonam Sodium
Cefaclor
Cefaloridin
Cefalotin Sodium
Cefamandole Lithium
Cefbuperazone
Cefmenoxime Hydrochloride
Cefodizime Sodium
Cefotaxime
Cefotetan
Cefotiam Hexetil Hydrochloride
Cefoxitin
Cefpiramide
Cefpodoxime Proxetil
Cefroxadine
Cefteram Pivoxil Mesitylenesulfonate
Cefuroxime Axetil
Chloramphenicol
Chloramphenicol Palmitate
Chloramphenicol Succinate
Ciclacillin
Clindamycin Hydrochloride
Clindamycin Phosphate
Colistin Sulfate
Daunorubicin Hydrochloride
Demethylchlortetracycline Hydrochloride
Dibekacin Sulfate
Diethanolamine Fusidate
Doxorubicin Hydrochloride
Doxycycline Hydrochloride
Enviomycin Sulfate
Epirubicin Hydrochloride
Flomoxef Triethylammonium
Fradiomycin Sulfate
Gentamicin Sulfate
Gramicidin
Griseofulvin
Imipenem
Josamycin Propionate
Kanamycin Monosulfate
Latamoxef Ammonium
Lenampicillin Hydrochloride
Lincomycin Hydrochloride
Lysozyme
Micronomicin Sulfate
Mitomycin C
Oxytetracycline Hydrochloride
Peplomycin Sulfate
L-Phenethicillin Potassium
Pimaricin
Pirarubicin
Pivmecillinam Hydrochloride
Polymixin B Sulfate
Puerarin
Pyrrolnitrin
Ranitidine Hydrochloride
Retinol Acetate
Retinol Palmitate
Ribostamycin Sulfate
Rifampicin
Sennoside A
Sennoside B
Siccanin
Spectinomycin Hydrochloride
Streptomycin Sulfate
Sulbenicillin Sodium
Talampicillin Hydrochloride
Tobramycin
Trichomycin
Vancomycin Hydrochloride

14. English and Latin titles of drugs are derived, in principle, from International Nonproprietary Names (INN) for Pharmaceutical Substances recommended by the World Health Organization. Japanese titles are derived from the Japanese version of this book. The chemical names are based on the rules of the International Union of Pure and Applied Chemis-

try (IUPAC).

15. Molecular formulas of organic compounds begin with C and then H, followed by other involved elements in the alphabetical order of the symbols of the elements.

16. Structural formulas of drugs represent, as far as possible, steric configurations.

17. Test procedures in monographs in Part I are, in principle, written in full even in corresponding monographs in Part II, and vice versa. The test procedures in monographs for preparations are also written in full even within the same part, except in the monographs for preparations having a corresponding monograph of their principal material substances.

18. In Official Monographs, names of some of the reagents and the test solutions were changed to the latest names based on the JIS.

19. The following articles were deleted from Official Monographs

Part I

Drostanolone Propionate
Drostanolone Propionate Injection
Floctafenine
Iopanoic Acid
Iopanoic Acid Tablets
Simfibrate
Sodium Iopodate
Sodium Iopodate Capsules

Part II

Phenovalin and Magnesium Oxide Powder
Compound Scopolia Extract and Tannic Acid Ointment
Compound Scopolia Extract and Tannic Acid Suppositories

20. The following articles were newly added to Official Monographs:

Part I

Aceglutamide Aluminum
Bacitracin
Benzylpenicillin Benzathine
Cefodizime Sodium
Cefpodoxime Proxetil
Chloramphenicol Palmitate
Chloramphenicol Sodium Succinate
Clindamycin Hydrochloride
Colistin Sulfate
Daunorubicin Hydrochloride
Demethylchlortetracycline Hydrochloride
Enviomycin Sulfate
Epirubicin Hydrochloride
Erythromycin Lactobionate
Etizolam
Gramicidin
Kanamycin Monosulfate
Ketotifen Fumarate
Kitasamycin Tartrate
Lenampicillin Hydrochloride
Lysozyme Hydrochloride
Ofloxacin
Phenethicillin Potassium
Pimaricin
Pirarubicin
Pyrrolnitrin
Ranitidine Hydrochloride
Siccanin
Sodium Fusidate
Spectinomycin Hydrochloride
Trimebutine Maleate

Part II

Alpinia Officinarum Rhizome
Asparagus Tuber
Burdock Fruit
Chrysanthemum Flower
Clematis Root
Eucommia Bark
Fritillaria Bulb
Gastrodia Tuber
Hemp Fruit
Jujube Seed
Loquat Leaf
Magnolia Flower
Notopterygium Rhizome
Polygonum Root
Uncaria Thorn

21. The following monographs were revised by an addition or a change in the Description or other items:

Part I

Acetylkitasamycin
Acetylspiramycin
Aclarubicin Hydrochloride
Actinomycin D
Amoxicillin
Ampicillin
Anhydrous Ampicillin
Ampicillin Sodium
Arbekacin Sulfate
Astromicin Sulfate
Bacampicillin Hydrochloride
Bekanamycin Sulfate
Benzbromarone
Benzylpenicillin Potassium
Betamethasone Sodium Phosphate

Betamethasone Valerate
Bisacodyl Suppositories
Bleomycin Hydrochloride
Bleomycin Sulfate
Calcium Chloride Injection
Calcium Polystyrene Sulfonate
d-Camphor
dl-Camphor
Carbazochrome Sodium Sulfonate
Carbidopa
Carumonam Sodium
Cefaclor
Cefaloridin
Cefalotin Sodium
Cefamandole Sodium
Cefbuperazone Sodium
Cefmenoxime Hydrochloride
Cefotaxime Sodium
Cefotetan
Cefotiam Hexetil Hydrochloride
Cefoxitin Sodium
Cefpiramide Sodium
Cefroxadine
Cefteram Pivoxil
Ceftibuten
Ceftriaxone Sodium
Cefuroxime Axetil
Cetraxate Hydrochloride
Chloramphenicol
Chlordiazepoxide Powder
Chlordiazepoxide Tablets
Chlormadinone Acetate
Chlorphenesin Carbamate
Ciclacillin
Citric Acid
Anhydrous Citric Acid
Clindamycin Phosphate
Clomifene Citrate
Cloxacillin Sodium
1% Codeine Phosphate Powder
10% Codeine Phosphate Powder
Codeine Phosphate Tablets
Dibekacin Sulfate
Diclofenamide
Diethylcarbamazine Citrate Tablets
1% Dihydrocodeine Phosphate Powder
10% Dihydrocodeine Phosphate Powder
Dihydroergotoxine Mesilate
Diltiazem Hydrochloride
Dipyridamole
Dobutamine Hydrochloride
Dopamine Hydrochloride Injection
Doxorubicin Hydrochloride
Doxycycline Hydrochloride
Droperidol
Enflurane
Ergocalciferol
Erythromycin
Estradiol Benzoate
Estriol
Famotidine for Injection
Flomoxef Sodium
Flurbiprofen
Folic Acid
Fradiomycin Sulfate
Gentamicin Sulfate
Glycerin
Concentrated Glycerin
Griseofulvin
Haloxazolam
Homochlorcyclizine Hydrochloride
Hydrochlorothiazide
Hydrocortisone Acetate
Hydrocortisone Sodium Phosphate
Idarubicin Hydrochloride
Idoxuridine Ophthalmic Solution
Imipenem
Indometacin Suppositories
Isoniazid Injection
Isoniazid Tablets
Josamycin
Josamycin Propionate
Kanamycin Sulfate
Kitasamycin
Latamoxef Sodium
Lincomycin Hydrochloride
Liothyronine Sodium Tablets
Magnesium Sulfate Injection
D-Mannitol Injection
Menatetrenone
Mepitiostane
Methotrexate
Meticrane
Micronomicin Sulfate
Migrenin
Minocycline Hydrochloride
Mitomycin C
Morphine Hydrochloride
Morphine Hydrochloride Injection
Morphine Hydrochloride Tablets
Norepinephrine
Norepinephrine Injection
Norgestrel and Ethinylestradiol Tablets
Oxytetracycline Hydrochloride
Peplomycin Sulfate
Pethidine Hydrochloride

Phytonadione
Pipemidic Acid Trihydrate
Piperacillin Sodium
Pivmecillinam Hydrochloride
Polymixin B Sulfate
Potassium Clavulanate
Povidone-Iodine
Prednisolone Acetate
Prednisolone Sodium Succinate for Injection
Primidone
Procaine Hydrochloride Injection
Procarbazine Hydrochloride
Pyrazinamide
Reserpine
Retinol Acetate
Retinol Palmitate
Ribostamycin Sulfate
Rifampicin
Roxithromycin
Scopolamine Butylbromide
Sisomicin Sulfate
Sodium Bicarbonate Injection
Sodium Chloride
10% Sodium Chloride Injection
Streptomycin Sulfate
Sulbenicillin Sodium
Talampicillin Hydrochloride
Tegafur
Teicoplanin
Theophylline
Thiamine Hydrochloride
Thiamine Nitrate
Thiopental Sodium
Tipepidine Hibenzate
Tobramycin
Tocopherol
Tocopherol Acetate
Triamcinolone
Trichomycin
Trimetoquinol Hydrochloride
Vancomycin Hydrochloride
Xylitol Injection

Part II

Acacia
Powdered Acacia
Aloe
Powdered Aloe
Areca
Asiasarum Root
Capsicum
Powdered Capsicum
Cinnamon Bark
Powdered Cinnamon Bark
Cod Liver Oil
Coptis Rhizome
Powdered Coptis Rhizome
Gardenia Fruit
Glycyrrhiza
Powdered Glycyrrhiza
Imperata Rhizome
Powdered Japanese Gentian
Magnolia Bark
Powdered Magnolia Bark
Morphine and Atropine Injection
Mulberry Bark
Opium Alkaloids Hydrochlorides
Powdered Panax Rhizome
Peony Root
Powdered Peony Root
Phellodendron Bark
Powdered Phellodendron Bark
Compound Phellodendron Powder for Cataplasm
Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder
Pueraria Root
Rhubarb
Powdered Rhubarb
Scopolia Extract and Ethyl Aminobenzoate Powder
Scutellaria Root
Powdered Scutellaria Root
Senna Leaf
Powdered Senna Leaf
Sinomenium Stem
Swertia Herb
Powdered Swertia Herb
Swertia and Sodium Bicarbonate Powder
Toad Venom
Vitamin A Oil
Vitamin A Oil Capsules

22. The monographs, which use in Identification the newly introduced Infrared Reference Spectra, are as follows:

Part I

Acetylspiramycin
Aclarubicin Hydrochloride
Ampicillin
Anhydrous Ampicillin
Ampicillin Sodium
Benzylpenicillin Benzathine
Benzylpenicillin Potassium
Bleomycin Hydrochloride
Bleomycin Sulfate
Carumonam Sodium
Cefaclor
Cefaloridin
Cefalotin Sodium

Cefamandole Sodium
Cefmenoxime Hydrochloride
Cefodizime Sodium
Cefotaxime Sodium
Cefotetan
Cefpodoxime Proxetil
Cefuroxime Axetil
Chloramphenicol
Chloramphenicol Sodium Succinate
Ciclacillin
Citric Acid
Anhydrous Citric Acid
Clindamycin Phosphate
Demethylchlortetracycline Hydrochloride
Doxorubicin Hydrochloride
Doxycycline Hydrochloride
Erythromycin
Etizolam
Flomoxef Sodium
Glycerin
Concentrated Glycerin
Griseofulvin
Homochlorcyclizine Hydrochloride
Imipenem
Ketotifen Fumarate
Kitasamycin Tartrate
Latamoxef Sodium
Lenampicillin Hydrochloride
Lincomycin Hydrochloride
Mitomycin C
Norepinephrine
Ofloxacin
Phenethicillin Potassium
Phytonadione
Pivmecillinam Hydrochloride
Pyrazinamide
Pyrrolnitrin
Ranitidine Hydrochloride
Rifampicin
Siccanin
Sodium Fusidate
Sulbenicillin Sodium
Talampicillin Hydrochloride
Theophylline
Trimebutine Maleate
Vancomycin Hydrochloride

23. The monographs, which use in Identification the newly introduced Ultraviolet-visible Reference Spectra, are as follows:

Part I

Acetylspiramycin
Aclarubicin Hydrochloride
Actinomycin D
Benzbromarone
Benzylpenicillin Benzathine
Benzylpenicillin Potassium
Bleomycin Hydrochloride
Bleomycin Sulfate
Carumonam Sodium
Cefaclor
Cefaloridin
Cefalotin Sodium
Cefamandole Sodium
Cefbuperazone Sodium
Cefmenoxime Hydrochloride
Cefodizime Sodium
Cefotaxime Sodium
Cefotetan
Cefotiam Hexetil Hydrochloride
Cefoxitin Sodium
Cefpiramide Sodium
Cefpodoxime Proxetil
Cefroxadine
Cefteram Pivoxil
Ceftriaxone Sodium
Cefuroxime Axetil
Chloramphenicol
Chloramphenicol Palmitate
Chloramphenicol Sodium Succinate
Cloxacillin Sodium
Daunorubicin Hydrochloride
Demethylchlortetracycline Hydrochloride
Doxorubicin Hydrochloride
Enviomycin Sulfate
Epirubicin Hydrochloride
Etizolam
Flomoxef Sodium
Gramicidin
Griseofulvin
Homochlorcyclizine Hydrochloride
Imipenem
Josamycin
Josamycin Propionate
Ketotifen Fumarate
Kitasamycin Tartrate
Latamoxef Sodium
Lysozyme Hydrochloride
Minocycline Hydrochloride
Mitomycin C
Norepinephrine
Ofloxacin
Oxytetracycline Hydrochloride
Peplomycin Sulfate
Phenethicillin Potassium
Pimaricin
Pirarubicin

Potassium Clavulanate
Pyrrolnitrin
Ranitidine Hydrochloride
Rifampicin
Siccanin
Theophylline
Trimebutine Maleate
Vancomycin Hydrochloride

24. The following monographs were revised in origin:

Part I

Acetylspiramycin
Aclarubicin Hydrochloride
Actinomycin D
Amoxicillin
Ampicillin
Anhydrous Ampicillin
Ampicillin Sodium
Arbekacin Sulfate
Astromicin Sulfate
Bacampicillin Hydrochloride
Bekanamycin Sulfate
Benzbromarone
Benzylpenicillin Potassium
Bleomycin Hydrochloride
Bleomycin Sulfate
Calcium Chloride Injection
Carumonam Sodium
Cefaclor
Cefaloridin
Cefalotin Sodium
Cefamandole Sodium
Cefbuperazone Sodium
Cefmenoxime Hydrochloride
Cefotaxime Sodium
Cefotetan
Cefotiam Hexetil Hydrochloride
Cefoxitin Sodium
Cefpiramide Sodium
Cefroxadine
Cefteram Pivoxil
Cefuroxime Axetil
Chloramphenicol
Ciclacillin
Citric Acid
Anhydrous Citric Acid
Clindamycin Phosphate
Dibekacin Sulfate
Doxorubicin Hydrochloride
Doxycycline Hydrochloride
Erythromycin
Flomoxef Sodium
Fradiomycin Sulfate
Gentamicin Sulfate
Glycerin
Concentrated Glycerin
Griseofulvin
Imipenem
Josamycin
Josamycin Propionate
Kanamycin Sulfate
Latamoxef Sodium
Lincomycin Hydrochloride
Micronomicin Sulfate
Mitomycin C
Oxytetracycline Hydrochloride
Peplomycin Sulfate
Pipemidic Acid Trihydrate
Pivmecillinam Hydrochloride
Polymixin B Sulfate
Potassium Clavulanate
Procarbazine Hydrochloride
Pyrazinamide
Retinol Acetate
Retinol Palmitate
Ribostamycin Sulfate
Rifampicin
Sodium Chloride
Streptomycin Sulfate
Sulbenicillin Sodium
Talampicillin Hydrochloride
Teicoplanin
Tobramycin
Trichomycin
Trimetoquinol Hydrochloride
Vancomycin Hydrochloride

Part II

Aloe
Cod Liver Oil
Evodia Fruit
Gardenia Fruit
Pueraria Root
Senna Leaf
Powdered Senna Leaf
Vitamin A Oil
Vitamin A Oil Capsules

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General Notices

Change the paragraph 6 to read:

6. The following abbreviations are used for the main units.

meter	m
centimeter	cm
millimeter	mm
micrometer	μm
nanometer	nm
kilogram	kg
gram	g
milligram	mg
microgram	μg
nanogram	ng
picogram	pg
Celsius degree	°C
square centimeter	cm^2
liter	L
milliliter	mL
microliter	μL
megahertz	MHz
per centimeter	cm^{-1}
newton	N
kilopascal	kPa
mole per liter	mol/L
millipascal second	mPa·s
square millimeter second	mm^2/s
lux	lx
mass per cent	%
mass parts per million	ppm
mass parts per billion	ppb
volume per cent	vol%
volume parts per million	vol ppm
mass per volume per cent	w/v%
hydrogen ion concentration	pH
Endotoxin unit	EU

Note: ''ppm'' used in the Nuclear Magnetic Resonance Spectroscopy (^1H) indicates the chemical shift, and ''w/v%'' is used in the formula or composition of preparations.

Change the paragraph 8 to read:

8. Standard temperature, ordinary temperature, room temperature, and lukewarm are defined as 20°C, 15 – 25°C, 1 – 30°C, and 30 – 40°C, respectively. A cold place, unless otherwise specified, shall be a place having a temperature of 1 – 15°C.

The temperatures of cold water, lukewarm water, warm water, and hot water are defined as not exceeding 10°C, 30 – 40°C, 60 – 70°C, and about 100°C, respectively.

The term ''heated solvent'' or ''hot solvent'' means a solvent heated almost to the boiling point of the solvent, and the term ''warmed solvent'' or ''warm solvent'' usually means a solvent heated to a temperature between 60°C and 70°C. The term ''heat on or in a water bath'' indicates, unless otherwise specified, heating with a boiling water bath or a steam bath at about 100°C.

Cold extraction and warm extraction are usually performed at temperatures of 15 – 25°C and 35 – 45 °C, respectively.

General Rules for Preparations

11. Injections

Change the paragraph (9) to read:

(9) Unless otherwise specified, Injections meet the requirements of the Sterility Test. In the case of drugs to be dissolved before use, carry out the test with the solution obtained by dissolving the contents in the attached solvent.

General Tests, Processes and Apparatus

Change to read:

General Tests, Processes and Apparatus includes common methods for tests and other articles related to them. Unless otherwise specified, the procedures for absorbance determination, absorbance ratio determination, acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, ammonium determination, arsenic determination, atomic absorption spectrophotometry, test for bacterial endotoxins, boiling point determination, distilling range determination, chloride determination, conductivity measurement, congealing point determination, test for content uniformity, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, endpoint detection in titrimetry, flame coloration, fluorometry, foreign insoluble matter test for injections, gas chromatography, heavy metals determination, infrared spectrophotometry, insoluble particulate matter test for injections, insoluble particulate matter test for ophthalmic solutions, iron determination, liquid chromatography, loss on drying determination, loss on ignition determination, mass variation test, melting point determination, methanol determination, methoxyl assay, test for microbial limit, test for microbial limit for crude drugs, microbiological potency determination for antibiotics, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolarity determination, oxygen flask combustion method, paper chromatography, particle size distribution test for preparations, pH determination, powder particle size determination, test for pyrogen, qualitative test, test for readily carbonizable substances, refractive index determination, residual solvents test, residue on ignition determination, specific gravity and density determination, specific surface area determination, test for sterility, sulfate determination, test for glass containers for injections, test for metal particles in ophthalmic ointments, test for plastic containers, test for rubber closure for aqueous infusions, test for total organic carbon, thermal analysis, thin-layer chromatography, viscosity determination, vitamin A assay, test for volatile contaminants in ethanol, water determination, and X-ray powder diffraction are performed as directed in the corresponding articles under the General Tests, Processes and Apparatus. The tests for melting point of fats, congealing point of fatty acids, specific gravity, acid value, saponification value, ester value, hydroxyl value, unsaponifiable matter and iodine value of fats and fatty oils are performed as directed in the corresponding items under the Fats and Fatty oils Test, and the tests for foreign matter and loss on drying, total ash, acid-insoluble ash, extract content, essential oil content of crude drugs are performed as directed in the corresponding items under the Crude Drugs Test.

23. Infrared Spectrophotometry

Change the Instrument and adjustment to read:

Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2850 cm^{-1} to the minimum at about 2870 cm^{-1} should be not less than 18% transmittance and that from the maximum at about 1583 cm^{-1} to the minimum at about 1589 cm^{-1} should be not less than 12% transmittance.

The wave number (cm^{-1}) scale is usually calibrated by the use of several characteristic absorption wave numbers (cm^{-1}) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

3060.0 ($\pm$1.5)	2849.5 ($\pm$1.5)	1942.9 ($\pm$1.5)
1601.2 ($\pm$1.0)	1583.0 ($\pm$1.0)	1154.5 ($\pm$1.0)
1028.3 ($\pm$1.0)		

When the dispersive infrared spectrophotometer is used, the permissible range of the absorption wave unmbers at 1601.2 cm^{-1} and at 1028.3 cm^{-1} should be both within $\pm$2.0 cm^{-1}.

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm^{-1}, and the difference of wave number should be withun 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1}.

Change to read:

52. Residue on Ignition Test

The Residue on Ignition Test is a method to measure the mass of the residual substance not volatilized when the sample is ignited with sulfuric acid by the method described be-

low. Generally, this test is intended for determining the content of inorganic substances contained as impurities in an organic substance.

The description, for example, ''not more than 0.10% (1 g),'' in a monograph, indicates that the mass of the residue is not more than 1.0 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and ''after drying'' indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying.

Procedure

Previously ignite a crucible of platinum, quartz or porcelain at 600 ± 50°C for 30 minutes, and weigh accurately after cooling in a desiccator (silica gel).

Take the sample of the amount directed in the monograph, transfer into the ignited crucible, and weigh accurately. When the quantity of the sample to be taken is indicated in a volume, pipet exactly the amount directed in the monograph and transfer into the above crucible. When directed as ''after evaporating,'' heat properly to evaporate the solution.

Moisten the sample with a small amount of sulfuric acid, usually 1 mL, then heat slowly at a temperature as low as practicable until the sample is completely carbonized, and cool. Moisten again with a small amount of sulfuric acid, heat gently until white fumes are no longer evolved, and ignite at 600 ± 50°C until the residue is completely incinerated. Proceed with care to not burn with a flame. Cool the crucible in a desiccator (silica gel), and reweigh accurately to calculate the amount of the residue.

When the amount of the residue obtained above exceeds the limit specified in the monograph, unless otherwise specified, ignite repeatedly to constant mass.

Change to read:

66. Vitamin A Assay

The Vitamin A Assay is a method to determine vitamin A in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. Method 1 is for the assay of synthetic vitamin A esters, using the ultraviolet-visible spectrophotometry (Method 1-1) or the liquid chromatography (Method 1-2). Method 2 is for the assay of vitamin A of natural origin, containing many geometrical isomers, using the ultraviolet-visible spectrophotometry to determine vitamin A as vitamin A alcohol obtained by saponification in an alkaline solution and extraction.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.300 μg of vitamin A (all-*trans* vitamin A alcohol).

Procedure

All procedures should be carried out quickly and care should be taken as far as possible to avoid exposure to light, air, oxidants, oxidizing catalysts (e.g. copper, iron), acids and heat. If necessary, light-resistant vessels may be used.

Generally, for synthetic vitamin A esters apply Method 1-1 or Method 1-2, but if the assay conditions required for Method 1-1 are not suitable, apply Method 2.

Method 1-1

Weigh accurately about 0.1 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 50 mL. Dilute this solution with 2-propanol for vitamin A assay to make a solution so that each mL contains 10 to 15 vitamin A Units, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution between 220 nm and 400 nm as directed under the Ultraviolet-visible Spectrophotometry to obtain the wavelength of the maximum absorption and the absorbances at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm. When the maximum absorption lies between 325 nm and 328 nm, and the ratios, $A_{\lambda i}/A_{326}$, of each absorbance, $A_{\lambda i}$, at 300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm to the absorbance, A_{326}, at 326 nm are within the range of ±0.030 of the values in the Table, the potency of vitamin A in Units per g of the sample is calculated from the following equation.

$$\text{Units of vitamin A in 1 g} = \frac{A_{326}}{W} \times \frac{V}{100} \times 1900$$

A_{326}: Absorbance at 326 nm
V: Total volume (mL) of the sample solution
W: Amount (g) of sample in V mL of the sample solution
1900: Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

This method is applied to drugs or preparations containing vitamin A esters (retinol acetate or retinol palmitate) as the main component. However, when the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the absorbance ratio $A_{\lambda i}/A_{326}$ is not within the range of ±0.030 of the values in the Table, apply Method 2.

Table Absorbance Ratio, $A_{\lambda i}/A_{326}$, of retinol acetate and retinol palmitate

λ_i (nm)	$A_{\lambda i}/A_{326}$	
	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

Method 1-2

Proceed with an appropriate amount of sample as directed under the Liquid Chromatography.

For the assay of retinol acetate and retinol palmitate use Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, respectively, and fix appropriately the operating procedure, the operating conditions and the system suitability based on the characteristics of the substance to be tested and the species and amount of coexisting substances.

Method 2

Unless otherwise specified, weigh accurately a sample containing not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL of a solution of potassium hydroxide (9 in 10), attach a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, transfer to a separator A, wash the flask with 10 mL of water and then 40 mL of diethyl ether, transfer the washings to the separator A, shake well, and allow to stand. Transfer the water layer so obtained to a separator B, wash the flask with 30 mL of diethyl ether, add the washing to the separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to the separator A, transfer the water layer in the flask to the separator B, add 30 mL of diethyl ether, and extract by shaking. Transfer the diethyl ether layer so obtained to the separator A, add 10 mL of water, allow the separator A to stand after gentle turning upside-down 2 or 3 times, and remove the water layer. Wash the content of the separator A with three 50-mL portions of water with increasingly vigorous shaking as the washing proceeds. Further wash with 50-mL portions of water until the washing no longer shows a pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, add 5 g of anhydrous sodium sulfate to the flask, mix by shaking, and transfer the diethyl ether to a round-bottomed flask by decantation. Wash the remaining sodium sulfate in the flask with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask. Evaporate the diethyl ether in a water bath at 45 °C while swirling the flask, using an aspirator, to about 1 mL, immediately add an exactly measured amount of 2-propanol for vitamin A assay to make a solution containing 6 to 10 vitamin A Units per mL, and designate the solution as the sample solution. Determine the absorbances, A_{310} at 310 nm, A_{325} at 325 nm, and A_{334} at 334 nm, of the sample solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Units of vitamin A in 1 g of the sample} = \frac{A_{325}}{W} \times \frac{V}{100} \times f \times 1830$$

$$f = 6.815 - 2.555 \times \frac{A_{310}}{A_{325}} - 4.260 \times \frac{A_{334}}{A_{325}}$$

A_{325}: Absorbance at 325 nm
V: Total volume (mL) of the sample solution
W: Amount (g) of sample in V mL of the sample solution
f: Correction factor
1830: Conversion factor from specific absorbance of retinol alcohol to IU (Unit/g)

70. Reference Standards; Reagents, Test Solutions; Standard Solutions for Volumetric Analysis; Standard Solutions; Matching Fluids for Color; Optical Filters for Wavelength and Transmission Rate Calibration; and Measuring Instruments, Appliances

(1) Reference Standards

Delete the following Reference Standards:

Drostanolone Propionate, Kitasamycin

Add the following:

Aceglutamide, Acetylspiramycin II, Aclarubicin, Actinomycin D, Arbekacin Sulfate, Astromicin Sulfate, Bacitracin, Bekanamycin Sulfate, Benzylpenicillin Potassium, Benzylpenicillin Sodium, Bleomycin A_2 Hydrochloride, Carumonam Sodium, Cefaclor, Cefaloridin, Cefalotin Sodium, Cefamandole Lithium, Cefbuperazone, Cefmenoxime Hydrochloride, Cefodizime Sodium, Cefotaxime, Cefotetan, Cefotiam Hexetil Hydrochloride, Cefoxitin, Cefpiramide, Cefpodoxime Proxetil, Cefroxadine, Cefteram Pivoxil Mesitylenesulfonate, Cefuroxime Axetil, Chloramphenicol, Chloramphenicol Palmitate, Chloramphenicol Succinate, Ciclacillin, Clindamycin Hydrochloride, Clindamycin Phosphate, Colistin Sulfate, Daunorubicin Hydrochloride, Demethylchlortetracycline Hydrochloride, Dibekacin Sulfate, Diethanolamine Fusidate, Doxorubicin Hydrochloride, Doxycycline Hydrochloride, Enviomycin Sulfate, Epirubicin Hydrochloride, Flomoxef Triethylammonium, Fradiomycin Sulfate, Gentamicin Sulfate, Gramicidin, Griseofulvin, Imipenem, Josamycin Propionate, Kanamycin Monosulfate, Latamoxef Ammonium, Lenampicillin Hydrochloride, Leucomycin A_5, Lincomycin Hydrochloride, Lysozyme, Micronomicin Sulfate, Mitomycin C, Oxytetracycline Hydrochloride, Peplomycin Sulfate, L-Phenethicillin Potassium, Pimaricin, Pirarubicin, Pivmecillinam Hydrochloride, Polymixin B Sulfate, Puerarin, Pyrrolnitrin, Ranitidine Hydrochloride, Retinol Acetate, Retinol Palmitate, Ribostamycin Sulfate, Rifampicin, Sennoside A, Sennoside B, Siccanin, Spectinomycin Hydrochloride, Streptomycin Sulfate, Sulbenicillin Sodium, Talampicillin Hydrochloride, Tobramycin, Trichomycin, Vancomycin Hydrochloride

(2) Reagents, Test Solutions

Delete the following Solution:

0.1 mol/L Phosphate buffer solution for ceftibuten, pH 8.0

Change the following:

Arecoline hydrobromide for thin-layer chromatography $C_8H_{13}NO_2$.HBr White crystals. Freely soluble in water, soluble in methanol, and practically insoluble in diethyl ether.

Melting point: 169 – 171°C

Purity Related substances—Dissolve 50 mg of arecoline hydrobromide for thin-layer chromatography in exactly 10 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Areca: any spot other than the principal spot at the *R*f value of about 0.4 does not appear.

***N*-Demethylroxithromycin** $C_{40}H_{74}N_2O_{15}$ White powder.

Identification—Determine the infrared absorption spectrum of a solution of the substance to be tested in chloroform (1 in 20) as directed in the solution method under the Infrared Spectrophotometry using a 0.1-mm cell made of potassium bromide: it exhibits absorption at the wave numbers of about 3600 cm^{-1}, 3520 cm^{-1}, 3450 cm^{-1}, 3340 cm^{-1}, 1730 cm^{-1} and 1627 cm^{-1}.

Geniposide for thin-layer chromatography $C_{17}H_{24}O_{10}$ White crystals or crystalline powder. Melting point: 159 – 163°C.

Purity Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20 μL of this solution as directed in the Identification (2) under Gardenia Fruit: any spot other than the principal spot at the *R*f value of about 0.3 does not appear.

Imidazole TS Dissolve 8.25 g of imidazole in 65 mL of water, adjust the pH to 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 100 mL.

Add the following:

2-Acetamidoglutarimide $C_7H_{10}N_2O_3$: 170.17

Identification—Determine the infrared absorption spectrum of 2-acetamidoglutarimide as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3350 cm^{-1}, 1707 cm^{-1}, 1639 cm^{-1} and 1545 cm^{-1}.

Purity Related substances—Dissolve 10 mg of 2-acetamidoglutarimide in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Proceed with 20 μL each of the sample solution and the standard solution as directed in the Purity (3) under Aceglutamide Aluminum: the total of the peak areas other than 2-acetamidoglutarimide from the sample solution is not more than the peak area from the standard solution.

Content: not less than 98.0%. Assay—Weigh accurately about 20 mg of 2-acetamidoglutarimide, and perform the test as directed under the Nitrogen Determination.

Each mL of 0.01 mol/L sulfuric acid VS
= 0.8509 mg of $C_7H_{10}N_2O_3$

Acetaminophen $C_8H_9NO_2$ [Same as the namesake monograph]

Acetate buffer solution, pH 5.4 To 5.78 mL of acetic acid (100) add water to make 1000 mL (solution A). Dissolve 8.2 g of anhydrous sodium acetate in water to make 1000 mL (solution B). Mix 176 mL of the solution A and 824 mL of the solution B, and adjust, if necessary, the pH to 5.4 with the solution A or the solution B.

0.02 mol/L Acetic acid-sodium acetate TS Dissolve 2.74 g of sodium acetate trihydrate in a suitable amount of water, and add 2 mL of acetic acid (100) and water to make 1000 mL.

Albiflorin $C_{23}H_{28}O_{11}.xH_2O$ Colorless powder having no odor. Freely soluble in water and in methanol, and practically insoluble in diethyl ether.

Purity—Dissolve 1 mg in diluted methanol (1 in 2) to make 10 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Peony Root; the total area of the peaks other than albiflorin and other than the solvent is not larger than 1/10 of the total area of the peaks other than the solvent peak.

0.05 mol/L Ammonium formate buffer solution, pH 4.0 Dissolve 3.5 g of ammonium formate in about 750 mL of water, adjust the pH to 4.0 with formic acid, and add water to make 1000 mL.

Amoxicillin $C_{16}H_{19}N_3O_5S.3H_2O$ [Same as the namesake monograph]

α-Apooxytetracycline $C_{22}H_{22}N_2O_8$ Yellow-brown to green powder.

Melting point: 200 – 205°C

β-Apooxytetracycline $C_{22}H_{22}N_2O_8$ Yellow-brown to brown powder.

Purity Related substances—Dissolve 8 mg of β-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than β-apooxytetracycline is not more than 10%.

L-Arabinose $C_5H_{10}O_5$ [K 8054: 1991, L(+)-Arabinose, Special class]

Aristolochic acid I for crude drugs purity test $C_{17}H_{11}NO_7$ Yellow crystalline powder. Melting point: about 280°C (with decomposition).

Absorbance $E^{1\%}_{1cm}$ (318 nm): 384 – 424 (1 mg, methanol, 100 mL).

Purity Related substances—Dissolve 1.0 mg of aristo-

lochic acid I for crude drugs purity test in 100 mL of diluted methanol (3 in 4), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aristolochic acid I obtained from the sample solution is not more than the peak area of aristolochic acid I from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3) under Asiasarum Root.

Time span of measurement: About 3 times as long as the retention time of aristolochic acid I after the solvent peak.

System suitability

Proceed as directed in the system suitability in the Purity (3) under Asiasarum Root.

Barbaloin for component determination Use barbaloin for thin-layer chromatography meeting the following additional specifications.

Absorbance $E^{1\%}_{1cm}$ (360 nm): 260 – 290 [10 mg dried in a desiccator (in vacuum, phosphorus (V) oxide) for not less than 24 hours, methanol, 500 mL].

Purity Related substances—Dissolve 10 mg of the substance to be tested in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20 μL each of the sample solution and the standard solution (1) as directed under the Liquid Chromatography according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than barbaloin from the sample solution is not larger than the peak area of barbaloin from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Component determination under Aloe except wavelength, detection sensitivity and time span of measurement.

Wavelength: 300 nm

Detection sensitivity: Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of barbaloin obtained from 20 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of barbaloin obtained from 20 μL of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of barbaloin after the solvent peak.

Becanamycin sulfate [Same as the namesake monograph]

Benzalphthalide $C_{15}H_{10}O_2$ Yellow crystalline powder. Melting point: 99 – 102°C.

4-Chlorobenzenediazonium TS Dissolve 0.5 g of 4-chloroaniline in 1.5 mL of hydrochloric acid, and add water to make 100 mL. To 10 mL of this solution add 10 mL of sodium nitrite TS and 5 mL of acetone. Prepare before use.

Cinnamic acid $C_9H_8O_2$ White crystalline powder, having a characteristic odor.

Melting point: 132 – 135°C

Citric acid-phosphate-acetonitrile TS Dissolve 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1).

***N*-Demethylerythromycin** $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

Deuterated formic acid for nuclear magnetic resonance spectroscopy DCOOD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated pyridine for nuclear magnetic resonance spectroscopy C_5D_5N Prepared for nuclear magnetic resonance spectroscopy.

Dibekacin sulfate [Same as the namesake monograph]

1,3-Dihydroxynaphthalene $C_{10}H_8O_2$ Purple-brown, crystals or powder. Freely soluble in water and in ethanol (95).

Melting point: about 125°C

***N*,*N*-Dimethylacetamide** $CH_3CON(CH_3)_2$ Clear and colorless liquid.

Boiling point: 163 – 165°C

Specific gravity: 0.938 – 0.945 (Method 3).

Water: not more than 0.2% (0.1 g, Coulometric titration).

Purity—Perform the test with 3 μL of *N*,*N*-dimethylacetamide as directed under the Gas Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of *N*,*N*-dimethylacetamide by the area percentage method: not less than 98.0%.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface 0.5 μm in thickness with polyethylene glycol 20 M for gas chromatography.

Column temperature: The sample is injected at a constant temperature of about 70°C, keep this temperature for 1 minute, then raise to 200°C in a rate of 10°C per minute, and keep 200°C for 3 minutes.

Carrier gas: Helium

Flow rate (linear velocity): About 30 cm/sec.

Time span of measurement: About 2 times as long as the retention time of *N*,*N*-dimethylacetamide.

System suitability

Test for required detection: To exactly 1.0 g of *N*,*N*-dimethylacetamide add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Confirm that the peak area of *N*,*N*-dimethylacetamide obtained from 3 μL of this solution is equivalent to 40 to 60% of the full-scale.

System repeatability: When the test is repeated with 3 μL of *N*,*N*-dimethylacetamide under the above operating conditions, the relative standard deviation of the peak area of *N*,*N*-dimethylacetamide is not more than 2.0%.

4-Dimethylaminoantipyrine $C_{13}H_{17}N_3O$ Colorless or white crystals, or a white crystalline powder.

Purity—Proceed the test with 5 μL of a solution of 4-dimethylaminoantipyrine (1 in 2000) as directed in the Assay under Cefpiramide Sodium, determine each peak area in a range of about 2 times as long as the retention time of 4-dimethylaminoantipyrine after the solvent peak by the automatic integration method, and calculate the total amount of the peaks other than 4-dimethylaminoantipyrine by the area percentage method: not more than 1.0%.

9,10-Diphenylanthracene $C_{26}H_{18}$ Yellow crystalline powder. Soluble in diethyl ether, and practically insoluble in water.

Melting point: about 248°C

1,4-Diphenylbenzene $C_{18}H_{14}$ White scaly crystals, having a slight aromatic odor. It is freely soluble in ethanol (99.5), and slightly soluble in water.

Identification—Determine the infrared absorption spectrum of 1,4-diphenylbenzene as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3050 cm^{-1}, 3020 cm^{-1}, 1585 cm^{-1}, 1565 cm^{-1}, 1476 cm^{-1}, 1450 cm^{-1}, 995 cm^{-1}, 834 cm^{-1}, 740 cm^{-1} and 680 cm^{-1}.

Divinylbenzene-methacrylate co-polymer for liquid chromatography Prepared for liquid chromatography.

6-Epidoxycycline hydrochloride $C_{22}H_{24}N_2O_8.HCl$ Yellow to dark yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of 6-epioxycycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of the peaks other than 6-epidoxycycline is not more than 10%.

4-Epioxytetracycline $C_{22}H_{24}N_2O_9$ Green-brown to brown powder.

Purity Related substances—Dissolve 20 mg of 4-epioxytetracycline in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than 4-epioxytetracycline is not more than 10%.

Erythromycin B $C_{37}H_{67}NO_{12}$ White to light yellowish white powder.

Purity Related substances—Dissolve 10 mg of erythromycin B in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μL each of the sample solution and the standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin B from the sample solution is not more than the peak area of erythromycin B from the standard solution.

Erythromycin C $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

Purity Related substances—Dissolve 10 mg of erythromycin C in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μL each of the sample solution and the standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin C from the sample solution is not more than the peak area of erythromycin C from the standard solution.

3-Ethoxy-4-hydroxybenzaldehyde $C_9H_{10}O_3$ White to pale yellowish white crystalline. Freely soluble in ethanol (95), and slightly soluble in water.

Melting point: 76 – 78°C

Content: not less than 98.0%. Assay—Weigh accurately about 0.3 g of 3-ethoxy-4-hydroxybenzaldehyde, previously dried in a desiccator (phosphorous (V) oxide) for 4 hours, dissolve in 50 mL of *N*,*N*-dimethylacetamide, and titrate with 0.1 mol/L sodium methoxide VS (indicator: thymol blue TS).

Each mL of 0.1 mol/L sodium methoxide VS
= 16.62 mg of $C_9H_{10}O_3$

2-Formylbenzoic acid $CHOC_6H_4COOH$ White crystals. Melting point: 97 – 99°C

Content: not less than 99.0%. Assay—Weigh accurately about 0.3 g of 2-formylbenzoic acid, previously dried (in vacuum, phosphorus (V) oxide, 3 hours), dissolve in 50 mL of freshly boiled and cooled water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.01 mg of $C_8H_6O_3$

Geniposide for component determination Use geniposide for thin-layer chromatography meeting the following additional specifications.

Absorbance $E^{1\%}_{1cm}$ (240 nm): 249 – 269 [10 mg dried in a desiccator (reduced pressure of not exceeding 0.67 kPa, phosphorus (V) oxide) for 24 hours, diluted methanol (1 in 2), 500 mL].

Purity Related substances—Dissolve 5 mg of geniposide for component determination in 50 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and the standard solution (1) as directed under the Liquid Chromatography according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than geniposide from the sample solution is not larger than the peak area of geniposide from the standard solution (1).

Operating conditions

Proceed as directed in the Component determination under Gardenia Fruit except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of geniposide obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of geniposide obtained from 10 μL of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of geniposide after the solvent peak.

Guaifenesin $C_{10}H_{14}O_4$ [Same as the namesake monograph]

Hirsutine $C_{22}H_{28}N_2O_3$ White to light yellow, crystals or crystalline powder. Melting point: about 100°C.

Optical rotation $[\alpha]_D^{20}$: about +57° (10 mg, methanol, 1 mL).

Absorbance $E^{1\%}_{1cm}$ (245 nm): 354 – 379 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances – Dissolve 5 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), proceed with 20 μL of this solution as directed in the Component determination under Uncaria Thorn, and perform the Liquid Chromatography: the sum of the peak areas except the areas of hirsutine and the solvent is not more than 1/10 of the sum of the peak areas except the solvent.

0.01 mol/L Hydrochloric acid-methanol TS To 20 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

0.05 mol/L Hydrochloric acid-methanol TS To 100 mL of 0.5 mol/L hydrochloric acid add methanol to make 1000 mL.

1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol $C_3H_6N_4OS$ White, crystals or powder.

Melting point: 136 – 141°C

Purity Related substances—Dissolve 0.10 g of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol in 1 mL of water, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, water, methanol and formic acid (60:10:7:6) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Isoniazid $C_6H_7N_3O$ [Same as the namesake monograph]

Josamycin $C_{42}H_{69}NO_{15}$ [Same as the namesake monograph]

Josamycin propionate $C_{45}H_{73}NO_{16}$ [Same as the namesake monograph]

Lactobionic acid $C_{12}H_{22}O_{12}$ Colorless crystals or white crystalline powder, having no odor.

Melting point: 113 – 118°C

Purity—Dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10 μL of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

Luteolin for thin-layer chromatography $C_{15}H_{10}O_6$ Light yellow to yellow-brown crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 310°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of luteolin for thin-layer chromatography in 1 mL of methanol. Proceed the test with 10 μL of this solution as directed in the Identification under Chrysanthemum Flower: any spot other than the principal spot of *R*f about 0.7 does not appear.

D-Mannose $C_6H_{12}O_6$ White crystal or crystalline powder. It is very soluble in water.

Melting point: about 132°C (with decomposition).

Optical rotation $[\alpha]_D^{20}$: +13.7 – +14.7° (4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm).

Metacycline hydrochloride $C_{22}H_{22}N_2O_8.HCl$ Yellow to dark yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of metacycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric

acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of peaks other than metacycline is not more than 10%.

1-Methyl-1*H*-tetrazole-5-thiol for liquid chromatography $C_2H_4N_4S$ White, crystals or crystalline powder. Very soluble in methanol, and freely soluble in water.

Melting point: 123 – 127°C

Loss on drying: not more than 1.0% (1 g, in vacuum, phosphorous (V) oxide, 2 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.2 g of 1-methyl-1*H*-tetrazole-5-thiol, previously dried, dissolve in 80 mL of *N*,*N*-dimethylformamide, and titrate with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-*N*,*N*-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 11.61 mg of $C_2H_4N_4S$

Morphine Hydrochloride [Same as the namesake monograph]

2-Naphthalenesulfonic acid $C_{10}H_8O_3S.H_2O$ White to pale yellowish white powder. Very soluble in water, in methanol and in ethanol (95), and sparingly soluble in diethyl ether and in chloroform.

Water: 7.0 – 11.5% (0.5 g, volumetric titration, direct titration).

Content: not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid, dissolve in 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L soduim hydroxide VS
= 20.82 mg of $C_{10}H_8O_3S$

1-Naphthol-sulfuric acid TS Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid, and mix well. Prepare before use.

Ninhydrin-acetic acid TS Dissolve 1.0 g of ninhydrin in 50 mL of ethanol (95), and add 10 mL of acetic acid (100).

Octadecylsilanized silica gel for thin-layer chromatography Octadecylsilanized silica gel prepared for thin-layer chromatography.

Octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography Octadecylsilanized silica gel for thin-layer chromatography containing fluorescent indicator.

Ofloxacin demethyl substance (±)-9-Fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-10-(1-piperazinyl)-pirido[1, 2, 3-*de*] [1, 4]benzoxazine-6-carboxylic acid $C_{17}H_{18}FN_3O_4$ White to light green-yellowish white, crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum of ofloxacin demethyl substance as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3050 cm^{-1}, 2840 cm^{-1}, 1619 cm^{-1}, 1581 cm^{-1}, 1466 cm^{-1}, 1267 cm^{-1}, 1090 cm^{-1}, 1051 cm^{-1} and 816 cm^{-1}.

Phenol red TS, dilute To 235 mL of a solution of ammonium nitrate (1 in 9400) add 105 mL of 2 mol/L sodium hydroxide TS and 135 mL of a solution prepared by dissolving 24 g of acetic acid (100) in water to make 200 mL. To this solution add 25 mL of a solution prepared by dissolving 33 mg of phenol red in 1.5 mL of 2 mol/L sodium hydroxide TS and adding water to make 100 mL. If necessary, adjust the pH to 4.7.

50% Phenyl-50% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

0.01 mol/L Phosphate buffer solution, pH 6.8 Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 6.8 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

0.03 mol/L Phosphate buffer solution, pH 7.5 Dissolve 4.083 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

1/15 mol/L Phosphate buffer solution, pH 5.6 Dissolve 9.07 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 5.6 with potassium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 5.3 Dissolve 0.44 g of disodium hydrogen phosphate 12-water and 13.32 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 5.3 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 6.8 Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate 12-water in about 750 mL of water, adjust the pH to 6.8 with sodium hydroxide TS if necessary, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution for antibiotics, pH 8.0 Dissolve 16.73 g of dipotassium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 8.0 with phosphoric acid, and add water to make 1000 mL.

Phosphate buffer solution, pH 3.1 Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water, and add 6.3 mL of phosphoric acid and water to make 1000 mL.

Phosphate buffer solution, pH 5.9 Dissolve 6.8 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 5.9 with diluted potassium hydroxide TS (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, pH 6.2 Dissolve 9.08 g of potassium dihydrogen phosphate in 1000 mL of water (solution A). Dissolve 9.46 g of disodium hydrogen phosphate in 1000 mL of water (solution B). Mix 800 mL of the solution A and 200 mL of the solution B, and adjust the pH to 6.2 with the solution A or the solution B if necessary.

Phosphate buffer solution for antibiotics, pH 6.5 Dissolve 10.5 g of disodium hydrogen phosphate 12-water and 5.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.5 with sodium hydroxide TS, and add water to make 1000 mL.

Phosphate TS Dissolve 2.0 g of dipotassium hydrogen phosphate and 8.0 g of potassium dihydrogen phosphate in water to make 1000 mL.

Phthalein purple $C_{32}H_{32}N_2O_{12}.xH_2O$ Yellowish white to brown power. Soluble in ethanol (95), and practically insoluble in water.

Sensitivity test—Dissolve 10 mg of phthalein purple in 1 mL of ammonia solution (28), and add water to make 100 mL. To 5 mL of this solution add 95 mL of water, 4 mL of ammonia solution (28), 50 mL of ethanol (95) and 0.1 mL of diluted barium chloride TS (1 in 5): the solution shows a blue-purple color which disappears on the addition of 0.15 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Phthalimide $C_8H_5NO_2$ White to pale brown crystals or powder.

Melting point: 232 – 237°C

Clarity—1.0 g of phthalimide dissolves in 20 mL of sodium hydroxide TS as a slight turbid solution.

Content: not less than 98.0%. Assay—Weigh accurately about 0.3 g of the substance to be tested, dissolve in 40 mL of *N*,*N*-dimethylformamide, and titrate with 0.1 mol/L sodium methoxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 14.71 mg of $C_8H_5NO_2$

Polyethylene glycol 600 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 1500 for gas chromatography Prepared for gas chromatography.

Polyoxyethylene (23) lauryl ether $C_{12}H_{25}(OCH_2CH_2)nOH$ White masses. Melting point: about 40°C

Potassium chloride for conductivity measurement [K 8121, Potassium chloride for conductivity measurement]

0.1 mol/L Potassium dihydrogen phosphate TS, pH 2.0 Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 2.0 with phosphoric acid.

0.25 mol/L Potassium dihydrogen phosphate TS, pH 3.5 Dissolve 34 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

0.33 mol/L Potassium dihydrogen phosphate TS Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 100 mL.

Potassium iodide TS, saturated Saturate 20 g of potassium iodide in 10 mL of fleshly boiled and cooled water. Prepare before use.

2-Propanol for vitamin A assay $(CH_3)_2CHOH$ [K 8839, Special class] When the absorbances at 300 nm and between 320 nm and 350 nm are determined as directed under the Ultraviolet-visible Spectrophotometry, using water as the control, they are not more than 0.05 and not more than 0.01, respectively. If necessary, purify by distillation.

Ranitidinediamine $(C_{10}H_{18}N_2OS)_2.C_4H_4O_4$ White to pale yellow crystalline powder.

Identification—Determine the infrared absorption spectrum of ranitidinediamine as directed in the paste method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 2780 cm^{-1}, 1637 cm^{-1}, 1015 cm^{-1} and 788 cm^{-1}.

Content: not less than 95%. Assay—Weigh accurately about 0.1 g of ranitidinediamine, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to green through blue (indicator: crystal violet TS). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.62 mg of $(C_{10}H_{18}N_2OS)_2.C_4H_4O_4$

Resibufogenin for thin-layer chromatography $C_{24}H_{32}O_4.nH_2O$ White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

Purity Related substances—Dissolve 5.0 mg of the substance to be tested in exactly 5 mL of acetone. Perform the test with 5 μL of this solution as directed in the Identification under Toad Venom: no other spots than the principal spot of around *R*f 0.4 appear.

L-Rhamnose monohydrate $C_6H_{12}O.H_2O$ White crystalline powder having sweet taste. Freely soluble in water, and sparingly soluble in ethanol (95).

Optical rotation $[\alpha]_D^{20}$: +7.8 – +8.3° (1 g, 20 mL of water, 2 drops of ammonia TS, 100 mm).

Melting point: 87 – 91°C

Purity Related substances—Dissolve 1.0 mg of L-rhamnose monohydrate in 1 mL of water, and add methanol to make exactly 10 mL. Proceed with 20 μL of this solution as directed in the Identification (2) under Acacia: any spot other than the principal spot at the *R*f value of about 0.5 does not appear.

Rhynchophylline for component determination $C_{22}H_{28}N_2O_4$ White, crystals or crystalline powder. Sparingly soluble in ethanol (99.5) and in acetone, and practically insoluble in water. Melting point: 205 – 209°C

Absorbance $E^{1\%}_{1cm}$ (245 nm): 473 – 502 (5 mg dried in a desiccator (silica gel) for 24 hours, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances—

(1) Dissolve 1.0 mg of rhynchophylline for component determination in 1 mL of acetone, and perform the test with this solution as directed under the Thin-layer Chromatography. Spot 10 μL of the solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of *R*f about 0.5 does not appear.

(2) Dissolve 5 mg of rhynchophylline for component determination in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area obtained from these solutions by the automatic integration method: the sum of the peak areas except the areas of rhynchophylline and the solvent obtained from the sample solution is not more than the peak area of rhynchophylline from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Component determination under Uncaria Thorn.

Time span of measurement: About 4 times as long as the retention time of rhynchophylline after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhynchophylline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the operating conditions in the Component determination under Uncaria Thorn.

Sodium dihydrogen phosphate TS, pH 2.5 Dissolve 2.7 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

6 mol/L Sodium hydroxide TS Dissolve 252 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

Sodium 1-methyl-1*H*-tetrazole-5-thiolate $C_2H_3N_4NaS.2H_2O$ White, crystals or crystalline powder.

Melting point: 90 – 94°C

Purity Related substances—Dissolve 10 mg of sodium 1-methyl-1*H*-tetrazole-5-thiolate in 10 mL of water, and use this solution as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Sodium 2-naphthalenesulfonate $C_{10}H_7NaO_3S$ Pale brown, crystals or powder.

Content: not less than 98.0%.

Sodium periodate TS Dissolve 60.0 g of sodium periodate in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. If the solution is not clear, filter this through a glass-filter. Keep in a light-resistant vessel.

Strongly acidic ion-exchange silica gel for liquid chromatography Prepared for liquid chromatography.

Substrate solution for lysozyme hydrochloride To a suitable amount of dried cells of *Micrococcus luteus* add a suitable amount of phosphate buffer solution, pH 6.2, gently shake to make a suspension, and add the substrate cells or the same buffer solution so that the absorbance of the suspension at 640 nm is about 0.65. Prepare before use.

Tetracycline Hydrochloride $C_{22}H_{24}N_2O_8.HCl$ Yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of tetracycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than tetracycline is not more than 10%.

Tetrafluoroethylene polymer for gas chromatography Prepared for gas chromatography.

Tetrahydrofuran for liquid chromatography C_4H_8O Clear and colorless liquid.

Refractive index n_D^{20}: 1.406 – 1.409

Density (20°C): 0.884 – 0.889 g/mL

Purity Ultraviolet absorbing substances—Determine the absorption spectrum of tetrahydrofuran for liquid chromatography as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank: the absorbences at 240 nm, 254 nm, 280 nm, 290 nm, and between 300 nm and 400 nm are not more than 0.35, 0.20, 0.05, 0.02 and 0.01, respectively.

Peroxide—Perform the test according to the method described in JIS K 9705: not more than 0.01%.

Tetrakishydroxypropylethylenediamine for gas chromatography Prepared for gas chromatography.

Tetra-*n*-propylammonium bromide
$[CH_3CH_2CH_2]_4NBr$ White, crystals or crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of tetra-*n*-propylammonium bromide in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Weigh accurately about 0.4 g of tetra-*n*-propylammonium bromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid, and titrate with 0.1 mol/L silver nitrate VS while shaking strongly (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 26.63 mg of $C_{12}H_{28}NBr$

Thymine $C_5H_6N_2O_2$: 126.11

Identification—Determine the infrared absorption spectrum of thymine, previously dried at 105°C for 3 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3030 cm^{-1}, 1734 cm^{-1}, 1676 cm^{-1}, 1446 cm^{-1} and 814 cm^{-1}.

Purity Related substances—Dissolve 50 mg of thymine in 100 mL of methanol. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Proceed with 10 μL of the sample solution as directed in the Purity (3) under Aceglutamide Aluminum: any peak does not appear at the retention time of aceglutamide.

Tin (II) chloride-hydrochloric acid TS To 20 g of tin add 85 mL of hydrochloric acid, heat until hydrogen gas no longer are evolved, and allow to cool. Mix 1 volume of this solution and 10 volume of dilute hydrochloric acid. Prepare before use.

Triethylamine buffer solution, pH 3.2 To 4 mL of triethylamine add 2000 mL of water, and adjust the pH to 3.2 with phosphoric acid.

Trimethylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Water, sterile purified [Same as the namesake monograph in Part II]

(3) Standard Solutions for Volumetric Analysis

Add the following:

Barium chloride, 0.1 mol/L

1000 mL of this solution contains 24.426 g of barium chloride dihydrate ($BaCl_2.2H_2O$: 244.26).

Preparation—Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of the prepared solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter the mixture, wash the precipitate on the filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat again at about 700°C for 2 hours. After cooling, weigh accurately the mass of the residue, and calculate the molarity factor as barium sulfate ($BaSO_4$).

Each mL of 0.1 mol/L barium chloride VS
= 23.34 mg of $BaSO_4$

Add the following:

72. Conductivity Measurement

Conductivity measurement is a method for the measuring the flowability of electric current in an aqueous solution. The measurement is made with a conductivity meter or a resistivity meter, and is used, for example, in the purity tests in monographs. The method is applied to evaluate the test item "Conductivity (Electrical Conductivity)" specified in the monographs. Further it is also used for monitoring the quality of water in the preparation of highly purified water. However, when applying this method for monitoring the quality of water, the details of measurement should be specified by the user, based on the principles described here.

Conductivity of a solution κ ($S \cdot m^{-1}$) is defined as the reciprocal of resistivity ρ ($\Omega \cdot m$), which is an indicator of the strength of ionic conductivity for a fluid conductor. Resistivity is defined as the product of electrical resistance per unit length and cross-sectional area of a conductor. When resistivity is ρ, cross-section area A (m^2), and length l (m), resistance R (Ω) can be expressed by the following equation.

$$R = \rho(l/A)$$

Thus, conductivity κ is expressed as follows,

$$\kappa = 1/\rho = (1/R)(l/A)$$

If l/A is known, the conductivity κ can be obtained by measuring resistance R or conductance G ($= R^{-1}$).

In the International System (SI), the unit of conductivity is the Siemens per meter ($S \cdot m^{-1}$). In practice, conductivity of a solution is generally expressed by $\mu S \cdot cm^{-1}$, and resistivity by $\Omega \cdot cm$.

Unless otherwise specified, the reference temperature for the expression of conductivity or resistivity is 20°C.

Apparatus

A conductivity meter or a resistivity meter is composed of an indicator part (operating panel, display, recording unit) and a detector part, the latter of which includes a conductivity cell. In the conductivity cell a pair of platinum electrodes is embedded. The cell is immersed in a solution, and the resistance or the resistivity of the liquid column between the electrodes is measured. Alternating current is supplied to this apparatus to avoid the effects of electrode polarization. Further, a temperature compensation system is generally

contained in the apparatus.

Conductivity measurement is generally performed by using an immersion-type cell. A pair of platinum electrodes, the surfaces of which are coated with platinum black, is fixed in parallel. Both electrodes are generally protected by a glass tube to prevent physical shocks.

When the surface area of the electrode is A (cm^2), and the separation distance of the two electrodes is l (cm), the cell constant C (cm^{-1}) is given by the following equation.

$$C = \alpha \cdot (l/A)$$

α is a dimensionless numerical coefficient, and it is characteristic of the cell design.

In addition to the immersion-type cell, there are flow-through-type and insert-in-pipe-type cells. These cells are set or inserted in an appropriate position in the flow system for monitoring the quality of water continuously or intermittently, during the preparation of highly purified water.

Standard Solution of Potassium Chloride

After pulverizing an appropriate amount of potassium chloride for conductivity measurement, dry it at 500 – 600°C for 4 hours. Take an indicated amount of the dried potassium chloride, as shown in Table 1, dissolve it in distilled or purified water (conductivity less than 2 $\mu S \cdot cm^{-1}$), previously boiled and cooled, and adjust to make 1000.0 g, for preparation of the standard solutions. The conductivity and the resistivity of the respective standard solutions at 20°C are indicated in Table 1. These standard solutions should be kept in tightly closed polyethylene or hard glass bottles.

Table 1. Conductivity and Resistivity of the Standard Solutions of Potassium Chloride at 20°C

Concentration (g/1000.0 g)	Conductivity κ ($\mu S \cdot cm^{-1}$)	Resistivity ρ ($\Omega \cdot cm$)
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

When measurement at 20°C can not be done, the indicated value of conductivity for the respective standard solution (Table 1), can be corrected by using the equation below. However, the equation is valid only within the range of 20 ± 5°C.

$$\kappa_T = \kappa_{20}[1 + 0.021(T - 20)]$$

T: Measuring temperature specified in the monograph
κ_T: Calculated conductivity of the KCl standard solution at T°C
κ_{20}: Conductivity of the KCl standard solution at 20°C

Operating Procedure

(1) Cell Constant

An appropriate conductivity cell should be chosen according to the expected conductivity of the sample solution. The higher the expected conductivity, the larger the cell constant required for the conductivity cell, so that the electrical resistance is within the measuring range of the apparatus being used. Conductivity cells with a cell constant of the order of 0.1 cm^{-1}, 1 cm^{-1}, or 10 cm^{-1}, are generally used.

For determination or confirmation of the cell constant, an appropriate KCl standard solution should be chosen and prepared, taking account of the expected conductivity of the sample solution to be measured. Rinse the cell several times with distilled water. Next, after rinsing the cell 2 – 3 times with the standard solution used for the cell constant determination, immerse the cell in the standard solution contained in a measuring vessel. After confirming that the temperature of the standard solution is maintained at 20 ± 0.1°C or at the temperature specified in the monograph, measure the resistance R_{KCl} or the conductance G_{KCl} of the standard solution, and calculate the cell constant C (cm^{-1}) by use of the following equation.

$$C = R_{KCl} \cdot \kappa_{KCl} \quad \text{or} \quad C = \kappa_{KCl}/G_{KCl}$$

R_{KCl}: Measured resistance (MΩ)
G_{KCl}: Measured conductance (μS)
κ_{KCl}: Conductivity of the standard solution being used ($\mu S \cdot cm^{-1}$)

The measured cell constant should be consistent with the given value within 5%. If it is not consistent, coat the electrodes with platinum black again, or replace the cell with a new one.

(2) Suitability Test for the Apparatus

Using an appropriate KCl standard solution according to the expected conductivity of the sample solution, perform the suitability test for the apparatus. Rinse the conductivity cell several times with distilled water, and rinse again 2 – 3 times with the selected standard solution. Fill the standard solution in the measuring vessel. After confirming that the temperature of the measuring system is maintained at 20 ± 0.1°C, measure the conductivity of the standard solution. When this measuring procedure is repeated several times, the average conductivity should be consistent with an indicated value in Table 1 within 5%. Further, the relative standard deviation should be less than 2%.

(3) Measurement

After confirmation of the suitability of the apparatus, perform the conductivity measurement for the sample solution. Unless otherwise specified, the preparation method for sample solution should be as specified in the respective monograph. Rinse the conductivity cell several times with distilled water, and rinse again 2 – 3 times with sample solution. Immerse the cell in the sample solution placed in a measuring vessel. If necessary, agitate gently the sample solution. After confirming that the temperature of the sample solution is maintained at 20 ± 0.1°C or at the temperature specified in the monograph, measure the resistance R_T (MΩ) or conductance G_T (μS) of the sample solution, and calculate the conductivity κ_T by using the following equation.

$$\kappa_T = CG_T \quad \text{or} \quad \kappa_T = C/R_T$$

Items such as the sample preparation method, the necessity of blank correction, the calculation method, the specification value, and the measuring temperature should be described in the monograph, if necessary.

Add the following:

73. Determination of Bulk and Tapped Densities

Determination of bulk and tapped densities is a method to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant. The bulk density is expressed in mass per unit apparent volume of powder (g/mL). Because the bulk density is one of the measures of packing properties, compressibility and flow properties, and is dependent on the ''history'' of the powder, it is essential to report the bulk density to specify how the determination was made.

Bulk density

The bulk density is an apparent density obtained by pouring a powder sample into a vessel without any consolidation. The determination of bulk density is achieved by measuring the apparent volume of a powder sample having a known mass in a graduated cylinder (Method 1) or by measuring the mass of powder in a vessel having a known volume (Method 2).

Method 1 (Constant mass method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000-μm (No.16) screen to break up agglomerates that may have formed during storage. Weigh accurately about 30 g of test sample, and pour it into a dry 100-mL graduated glass cylinder (readable to 1 mL). Carefully level the powder without consolidation, if necessary, and read the unsettled apparent volume, V_0, to the nearest graduated unit. Calculate the bulk density ρ_B by the formula:

$$\rho_B = \frac{M}{V_0}$$

ρ_B: Bulk density by constant mass method (g/mL)
M: Mass of powder sample (g)
V_0: Apparent volume of powder sample (mL)

Record the average of 3 determinations using 3 different powder samples. If a 30-g sample is too large to determine, adjust the mass of sample so as to provide an apparent volume of 60 – 100 mL.

Method 2 (Constant volume method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000-μm (No.16) screen to break up agglomerates that may have formed during storage. Allow an excess of sample powder to pour into the measuring vessel having the volume of V and mass of M_0. Carefully scrape excess powder from the top of the vessel by smoothly moving across it the edge of a slide glass or other tool. Remove any material from the sides of the vessel, and determine the total mass M_t. Calculate the bulk density ρ_B by the formula:

$$\rho_B = \frac{M_t - M_0}{V}$$

ρ_B: Bulk density by constant volume method (g/mL)
M_t: Total mass of powder and measuring vessel (g)
M_0: Mass of measuring vessel (g)
V: Volume of measuring vessel (mL)

Record the average of 3 determinations using 3 different powder samples.

Tapped density

Tapped density is an apparent density obtained by mechanically tapping a measuring vessel containing a powder sample. The determination of tapped density is achieved by measuring the apparent volume of a powder sample having a known mass in a vessel after tapping (Method 1) or by measuring the mass of powder in a vessel having a known volume after tapping (Method 2).

Method 1 (Constant mass method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000-μm (No.16) or a 710-μm (No.22) screen to break up agglomerates that may have formed during storage. Weigh accurately about 100 g of test sample, and pour it into a 250-mL graduated glass cylinder (readable to 2 mL) without consolidation. If it is not possible to use 100 g, proceed according to the same procedure as that described above by using a 100-mL graduated glass cylinder (readable to 1 mL). It is essential to select appropriate masses of the cylinder support, holder and cylinder so as to ensure the dynamic stability of the apparatus during tapping. After attaching the glass cylinder containing the powder sample to the tapping apparatus, carry out tapping under the measuring conditions (tapping rate and drop height) specified for each apparatus.

Unless otherwise specified, repeat increments of 50 taps or 1 minute until the difference between succeeding measurements is less than 2%, and determine the final apparent volume, V_f. Calculate the tapped density ρ_T by the formula:

$$\rho_T = \frac{M}{V_f}$$

ρ_T: Tapped density by constant mass method (g/mL)
M: Mass of powder sample (g)
V_f: Final apparent volume of sample after tapping (mL)

Record the average of 3 determinations using 3 different powder samples.

Method 2 (Constant volume method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000-μm (No.16) screen to break up agglomerates that may have formed during storage. Attach a supplementary cylinder to the stainless steel measuring vessel having a known mass of M_0 and a volume of V (Fig. 1), and then pour an excess of the sample into the vessel. After setting up the vessel in an adequate tap-

ping apparatus with a fixed drop height, carry out tapping at the rate and cumulative tap number specified for each apparatus. Then remove the supplementary cylinder from the vessel and carefully scrape excess powder from the top of the vessel by smoothly moving across it the edge of a slide glass or other tool. Remove any material from the sides of the vessel, and determine the total mass M_t. Calculate the tapped density ρ_T by the formula:

$$\rho_T = \frac{M_t - M_0}{V}$$

ρ_T: Tapped density by constant volume method (g/mL)

M_t: Total mass of powder and measuring vessel (g)
M_0: Mass of measuring vessel (g)
V: Volume of measuring vessel (mL)

Record the average of 3 determinations and the relative standard deviation using 3 different powder samples. If the relative standard deviation is not less than 2%, repeat the test with further tapping.

Balances: Use balances readable to the nearest 0.1 g.

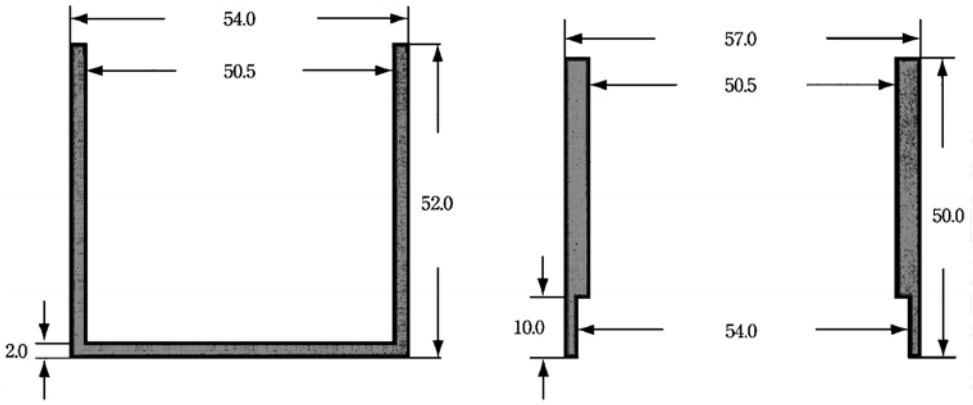


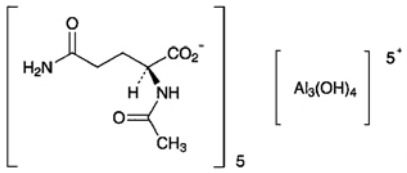
Fig. 1 Measuring vessel (left) and supplementary cylinder (right)

Official Monographs for Part I

Add the following:

Aceglutamide Aluminum

アセグルタミドアルミニウム



$C_{35}H_{59}Al_3N_{10}O_{24}$: 1084.84
Pentakis[(2*S*)-2-acetylamino-4-carbamoylbutanoato]tetrahydroxotrialuminium
[*12607-92-0*]

Aceglutamide Aluminum contains not less than 85.4% and not more than 87.6% of aceglutamide ($C_7H_{12}N_2O_4$: 188.18), and not less than 7.0% and not more than 8.0% of aluminum (Al: 26.98), calculated on the dried basis.

Description Aceglutamide Aluminum occurs as a white powder, having astringent bitter taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification **(1)** Dissolve 0.03 g each of Aceglutamide Aluminum and Aceglutamide Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of bromocresol green in ethanol (95) (1 in 1000), then spray evenly diluted ammonia solution (28) (1 in 100): the spots from the sample solution and the standard solution show a light yellow and have the same *R*f value.

(2) A solution of Aceglutamide Aluminum in dilute hydrochloric acid (1 in 20) responds to the Qualitative Tests for aluminum salt.

Optical rotation $[\alpha]_D^{20}$: -5.5 – $-7.5°$ (2 g calculated on the dried basis, water, 50 mL, 100 mm).

Purity **(1)** Heavy metals—Put 1.0 g of Aceglutamide Aluminum in a porcelain crucible, cover the crucible loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat in the same manner as above, then ignite at 500 to 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution with the same amount of the reagents, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Aceglutamide Aluminum according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Aceglutamide Aluminum in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-acetamidoglutarimide in the mobile phase to make exactly 100 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, the standard solution (1) and the standard solution (2) as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 2-acetamidoglutarimide from the sample solution is not more than that from the standard solution (2), the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution are not more than 3/10 times the peak area of aceglutamide from the standard solution (1), and the total of the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution is not more than the peak area of aceglutamide from the standard solution (1).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of aceglutamide.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution (1) add the mobile phase to make exactly

50 mL. Confirm that the peak area of aceglutamide obtained from 20 μL of this solution is equivalent to 7 to 13% of that of aceglutamide obtained from 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of aceglutamide is not more than 2.0%.

Loss on drying Not more than 5.0% (1 g, 130°C, 5 hours).

Assay (1) Aceglutamide—Weigh accurately about 50 mg of Aceglutamide Aluminum, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Aceglutamide Reference Standard, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of aceglutamide to that of the internal standard.

$$\text{Amount (mg) of aceglutamide } (C_7H_{12}N_2O_4) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Aceglutamide Reference Standard

Internal standard solution—A solution of thymine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted perchloric acid (1 in 1000) and methanol (99:1).

Flow rate: Adjust the flow rate so that the retention time of aceglutamide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aceglutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aceglutamide to that of the internal standard is not more than 1.0%.

(2) Aluminum—Weigh accurately about 3.0 g of Aceglutamide Aluminum, add 20 mL of dilute hydrochloric acid, and heat on a water bath for 60 minutes. After cooling, add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

Containers and storage Containers—Tight containers.

Acetylkitasamycin

アセチルキタサマイシン

Change the Assay (3) to read:

Assay

(3) Standard solution－Weigh accurately an amount of Leucomycin A_5 Reference Standard equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

Acetylspiramycin

アセチルスピラマイシン

Change to read except the structural formula and chemical name:

Acetylspiramycin contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Acetylspiramycin is expressed as mass (potency) of acetylspiramycin II ($C_{47}H_{78}N_2O_{16}$: 927.13). 1 mg (potency) of Acetylspiramycin is equivalent to 0.7225 mg of acetylspiramycin II ($C_{47}H_{78}N_2O_{16}$).

Description Acetylspiramycin occurs as a white to light yellowish white powder.

It is very soluble in acetonitrile and in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Acetylspiramycin in methanol (1 in 50,000) as

directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acetylspiramycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Content ratio of the active principle Dissolve 25 mg of Acetylspiramycin in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the areas, A_{II}, A_{III}, A_{IV}, A_V, A_{VI} and A_{VII}, of the peaks of acetylspiramycin II, acetylspiramycin III, acetylspiramycin IV, acetylspiramycin V, acetylspiramycin VI and acetylspiramycin VII, respectively, by the automatic integration method, and calculate the ratios of the amounts of A_{II}, A_{IV} and the total of A_{III} and A_V to the total amount of all these peaks: the amount of A_{II} is 30 – 45%, A_{IV} is 30 – 45%, and the total of A_{III} and A_V is not more than 25%. The relative retention times of acetylspiramycin III, acetylspiramycin IV, acetylspiramycin V, acetylspiramycin VI and acetylspiramycin VII with respect to acetylspiramycin II are 1.3, 1.7, 2.3, 0.85 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS and a solution of dipotassium hydrogen phosphate (87 in 25,000) (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of acetylspiramycin II is about 10 minutes.

System suitability—

System performance: Dissolve 25 mg of Acetylspiramycin II Reference Standard in the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of acetylspiramycin II are not less than 14,500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of acetylspiramycin II is not more than 2.0%.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Acetylspiramycin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 2.0 g of Acetylspiramycin according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying Not more than 3.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition Not more than 0.50% (1.0 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Acetylspiramycin II Reference Standard, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Acetylspiramycin, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Aclarubicin Hydrochloride

塩酸アクラルビシン

Change to read except the structural formula and chemical name:

Aclarubicin Hydrochloride contains not less than 860 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aclarubicin Hydrochloride is expressed as mass (potency) of aclarubicin ($C_{42}H_{53}NO_{15}$: 811.87).

Description Aclarubicin Hydrochloride occurs as a yellow to pale orange-yellow powder.

It is very soluble in chloroform and in methanol, freely soluble in water, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Aclarubicin Hydrochloride in diluted methanol (4 in 5) (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aclarubicin Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Aclarubicin Hydrochloride in methanol (1 in 200) responds to the Qualitative Test (2) for chloride.

Optical rotation $[\alpha]_D^{20}$: -146 – $-162°$ (0.05 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.05 g of Aclarubicin Hydrochloride in 10 mL of water is between 5.5 and 6.5.

Purity **(1)** Clarity and color of solution – Dissolve 0.10 g of Aclarubicin Hydrochloride in 10 mL of water: the solution is clear and yellow to pale orange-yellow.

(2) Heavy metals—Proceed with 1.0 g of Aclarubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Aclarubicin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the area percentage method: the amount of aklavinone having the relative retention time of about 0.6 to aclarubicin is not more than 0.2%, aclacinomycin L1 having the relative retention time of about 0.75 to aclarubicin is not more than 0.5%, 1-deoxypyrromycin having the relative retention time of about 1.7 to aclarubicin is not more than 1.5% and aclacinomycin S1 having the relative retention time of about 2.3 to aclarubicin is not more than 0.5%, and the total amount of the peaks other than aclarubicin and the peaks mentioned above is not more than 1.0% of the peak area of aclarubicin.

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of chloroform, methanol, acetic acid (100), water and triethylamine (6800:2000:1000:200:1).

Flow rate: Adjust the flow rate so that the retention time of aclarubicin is about 5 minutes.

Time span of measurement: As long as about 4 times of the retention time of aclarubicin after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aclarubicin obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Aclarubicin Hydrochloride in 10 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand for 60 minutes. To 1.0 mL of this solution add 1.0 mL of 0.2 mol/L sodium hydroxide TS, 1.0 mL of phosphate buffer solution, pH 8.0 and 1.0 mL of chloroform, shake vigorously, and take the chloroform layer. When the procedure is run with 20 μL of the chloroform under the above operating conditions, aclarubicin and 1-deoxypyrromycin are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 5 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of aclarubicin is not more than 2.0%.

Water Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aclarubicin Hydrochloride, equivalent to about 20 mg (potency), and dissolve in diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aclarubicin Reference Standard, equivalent to about 20 mg (potency), add 0.6 mL of diluted hydrochloric acid (1 in 250) and diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry, and determine the absorbencies, A_T and A_S, at 433 nm.

$$\text{Amount [}\mu\text{g (potency)] of aclarubicin } (C_{42}H_{53}NO_{15}) = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Aclarubicin Reference Standard

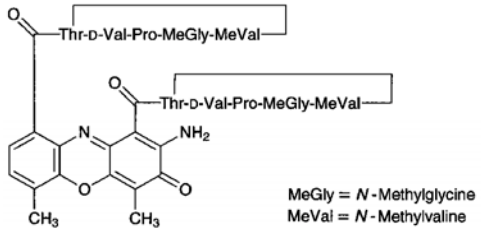
Containers and storage Containers—Tight containers.

Storage—Light-resistant and at 5°C or below.

Change to read:

Actinomycin D

アクチノマイシン**D**



$C_{62}H_{86}N_{12}O_{16}$: 1255.42
[*50-76-0*]

Actinomycin D, when dried, contains not less than 950 μg (potency) per mg. The potency of Actinomycin D is expressed as mass (potency) of actinomycin D ($C_{62}H_{86}N_{12}O_{16}$).

Description Actinomycin D occurs as an orange-red to red crystalline powder.

It is freely soluble in acetone, sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Actinomycin D in methanol (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Actinomycin D Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Actinomycin D and Actinomycin D Reference Standard in 10 mL of acetone, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and methanol (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spot from the sample solution is the same as that from the standard solution.

Optical rotation $[\alpha]_D^{20}$: -292 – $-317°$ (after drying, 10 mg, methanol, 10 mL, 100 mm).

Loss on drying Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Actinomycin D and Actinomycin D Reference Standard, previously dried, equivalent to about 60 mg (potency), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 25 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak area of actinomycin D, A_T and A_S, of both solutions.

$$\text{Amount [}\mu\text{g (potency)] of } C_{62}H_{86}N_{12}O_{16} = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Actinomycin D Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L acetic acid-sodium acetate TS and acetonitrile (25:23).

Flow rate: Adjust the flow rate so that the retention time of actinomycin D is about 23 minutes.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetrical coefficient of the peak of actinomycin D are not less than 2000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of actinomycin D is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Amoxicillin

アモキシシリン

Change the origin/limits of content to read:

Amoxicillin contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Amoxicillin is expressed as mass (potency) of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Add the following next to Identification:

Optical rotation $[\alpha]_D^{20}$: $+290$ – $+315°$ (0.1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Add the following next to Purity (2):

Purity

(3) Related substances—Dissolve 0.10 g of Amoxicillin

in 50 mL of a solution of sodium tetraborate decahydrate (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than amoxicillin obtained from the sample solution is not more than the peak area of amoxicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of amoxicillin.

System suitability—

Test for required detection: To exactly 1 mL of the standard solution add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that of amoxicillin obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Change the Assay to read:

Assay Weigh accurately an amount of Amoxicillin and Amoxicillin Reference Standard, equivalent to about 30 mg (potency), dissolve each in a solution of boric acid (1 in 200) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the peak areas, A_T and A_S, of amoxicillin of each solution.

$$\text{Amount [}\mu\text{g (potency)] of } C_{16}H_{19}N_3O_5S = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Amoxicillin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Ampicillin

アンピシリン

Change to read except the structural formula and chemical name:

Ampicillin contains not less than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Ampicillin occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Ampicillin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Ampicillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Ampicillin in 400 mL of water is between 3.5 and 5.5.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Ampicil-

lin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Ampicillin in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than that of ampicillin obtained from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

(4) *N*,*N*-Dimethylaniline—Weigh accurately about 1 g of Ampicillin, dissolve in 5 mL of sodium hydroxide TS, add exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the sample solution. Separately, weigh accurately about 50 mg of *N*,*N*-dimethylaniline, dissolve in 2 mL of hydrochloric acid and 20 mL of water, add water to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, determine the ratios, Q_T and Q_S, of the peak area of *N*,*N*-dimethylaniline to that of the internal standard, and calculate the amount of *N*,*N*-dimethylaniline by the following equation: not more than 20 ppm.

$$\text{Amount (ppm) of } N,N\text{-dimethylaniline} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times 400$$

W_S: Amount (g) of *N*,*N*-dimethylaniline

W_T: Amount (g) of the sample

Internal standard solution—A solution of naphthalene in cyclohexane (1 in 20,000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with 50% phenyl-50% methyl polysiloxane for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 120°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of *N*,*N*-dimethylaniline is about 5 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand for the test. Confirm that when the procedure is run with 1 μL of the upper layer liquid under the above operating conditions, the ratio of the peak area of *N*,*N*-dimethylaniline to that of the internal standard is equivalent to 15 – 25% of the ratio of the peak area of *N*,*N*-dimethylaniline to that of the internal standard obtained from the standard solution.

System performance: Dissolve 50 mg of *N*,*N*-dimethylaniline in cyclohexane to make 50 mL. To 1 mL of this solution add the internal standard solution to make 50 mL, and use this solution as the solution for system performance test. When the procedure is run with 1 μL of the solution for system performance test under the above operating conditions, *N*,*N*-dimethylaniline and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system performance test under the above operating conditions, the relative standard deviation of the ratios of the peak area of *N*,*N*-dimethylaniline to that of the internal standard is not more than 2.0%.

Water 12.0 – 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ampicillin and Ampicillin Reference Standard, equivalent to about 50 mg (potency), dissolve in a suitable volume of the mobile phase, add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{16}H_{19}N_3O_4S = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Ampicillin Reference

Standard

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Anhydrous Ampicillin

無水アンピシリン

Change to read except the structural formula and chemical name:

Anhydrous Ampicillin contains not less than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Anhydrous Ampicillin is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$).

Description Anhydrous Ampicillin occurs as white to light yellowish white, crystals or crystalline powder. It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Anhydrous Ampicillin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Anhydrous Ampicillin in 100 mL of water is between 4.0 and 5.5.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Anhydrous Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Anhydrous Ampicillin in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than ampicillin from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: As long as about 10 times of the retention time of ampicillin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water Not more than 2.0% (2.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Anhydrous Ampicillin and Ampicillin Reference Standard, equivalent to about 50 mg (potency), add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Ampicillin Reference Standard

Internal standard solution—A solution of guaifenesin in the

mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1%.

Containers and storage Containers—Tight containers.

Ampicillin Sodium

アンピシリンナトリウム

Change to read except the structural formula and chemical name:

Ampicillin Sodium contains not less than 850 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Sodium is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Ampicillin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is very soluble in water, and sparingly soluble in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Ampicillin Sodium, previously dried in a desiccator (reduced pressure not exceeding 0.67 kPa, 60°C) for 3 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Ampicillin Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +246 – +272° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Ampicillin Sodium in 10 mL of water is between 8.0 and 10.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ampicillin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Ampicillin Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than ampicillin obtained from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ampicillin obtained from 10 μL of the standard solution.

System performance: Dissolve 50 mg of Ampicillin Reference Standard in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200) and the mobile phase to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the

resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of guaifenesin is not more than 1.0%.

Water Not more than 2.0% (0.2 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 6.5 to 6.6 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 5 μg (potency) and 1.25 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Ampicillin Sodium, equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 5 μg (potency) and 1.25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Arbekacin Sulfate

硫酸アルベカシン

Change to read except the structural formula and chemical name:

Arbekacin Sulfate contains not less than 670 μg (potency) per mg, calculated on the dried basis. The potency of Arbekacin Sulfate is expressed as mass (potency) of arbekacin ($C_{22}H_{44}N_6O_{10}$: 552.62).

Description Arbekacin Sulfate occurs as a white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 10 mg each of Arbekacin Sulfate and Arbekacin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot from the standard solution are purple-brown in color and their *R*f values are the same.

(2) A solution of Arbekacin Sulfate (1 in 50) responds to the Qualitative Test (1) for sulfate.

Optical rotation $[\alpha]_D^{20}$: +69 – +79° (0.25 g after drying, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.75 g of Arbekacin Sulfate in 10 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Arbekacin Sulfate in 5 mL of water is clear and colorless.

(2) Heavy metals—Proceed with 2.0 g of Arbekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Dibekacin—Weigh accurately about 20 mg of Arbekacin Sulfate, add exactly 10 mL of the internal standard solution to dissolve, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Dibekacin Sulfate Reference Standard, equivalent to about 10 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of dibekacin to that of the internal standard. Calculate the amount of dibekacin by the following equation: not more than 2.0%.

$$\text{Amount (\%) of dibekacin} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times \frac{1}{50} \times 1000$$

W_S: Amount [mg (potency)] of Dibekacin Sulfate Reference Standard

W_T: Amount (mg) of the sample

Internal standard solution—A solution of bekanamycin sulfate (1 in 2000).

Operating conditions—

Detector: Fluorometry (excitation wavelength: 340 nm, detection wavelength: 460 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Reaction coil: A column about 0.3 mm in inside diameter

and about 3 m in length.

Reaction coil temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8.70 g of sodium 1-pentane sulfonate and 8.52 g of anhydrous sodium sulfate in 980 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 230 mL of this solution add 20 mL of methanol.

Reagent: Dissolve 12.36 g of boric acid in 960 mL of water, add 10 mL of a solution of *o*-phthalaldehyde in ethanol (99.5) (1 in 25), adjust the pH to 10.5 with 8 mol/L potassium hydroxide TS, and add water to make 1000 mL. To this solution add 1 mL of 2-mercaptoethanol.

Reaction temperature: A constant temperature of about 50°C.

Flow rate of the mobile phase: 0.5 mL per minute.

Flow rate of the reagent: 1 mL per minute.

System suitability—

System performance: Dissolve 20 mg each of Arbekacin Sulfate, becanamycin sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, becanamycin, arbekacin and dibekacin are eluted in this order, and the resolution between the peaks, becanamycin and arbekacin is not less than 5 and arbekacin and dibekacin is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dibekacin to that of the internal standard is not more than 2.0%.

(4) Related substances—Dissolve 20 mg of Arbekacin Sulfate in 20 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than arbekacin and dibekacin obtained from the sample solution is not more than the peak area of arbekacin from the standard solution.

Operating conditions—

Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reagent, reaction temperature, flow rate of mobile phase, and flow rate of reagent: Proceed as directed in the operating conditions in the Purity (3).

Time span of measurement: About 1.5 times as long as the retention time of arbekacin.

System suitability—

System performance: Dissolve 10 mg each of Arbekacin Sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, arbekacin and dibekacin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbekacin is not more than 5.0%.

Loss on drying Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organisms [5] under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Arbekacin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Arbekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Astromicin Sulfate

硫酸アストロマイシン

Change to read except the structural formula and chemical name:

Astromicin Sulfate contains not less than 606 μg (potency) per mg, calculated on the anhydrous basis. The potency of Astromicin Sulfate is expressed as mass (potency) of astromicin ($C_{17}H_{35}N_5O_6$: 405.49).

Description Astromicin Sulfate occurs as a white to light yellowish white, powder or masses.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 10 mg each of Astromicin Sulfate and Astromicin Sulfate Reference Standard in 10 mL of water. To 5 mL each of these solutions add water to make

100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Liquid Chromatography according to the following conditions: the retention time of astromicin obtained from the sample solution is the same with that from the standard solution.

Operating conditions—

Detector, column, column temperature, reaction coil, temperature of reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Purity (3).

(2) To 2 mL of a solution of Astromicin Sulfate (1 in 100) add 2 to 3 drops of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation $[\alpha]_D^{20}$: +90 – +110° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Astromicin Sulfate in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Astromicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Astromicin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Astromicin Sulfate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the related substance III, having the relative retention time of about 0.1, and the related substance I, having the relative retention time of about 1.2 with respect to the peak of astromicin, from the sample solution are not more than the peak area of astromicin from the standard solution, the peak area of the related substance II, having the related retention time of about 0.8, is not more than 2.0 times the peak area of astromicin from the standard solution, and the total area of the peaks other than astromicin from the sample solution is not more than 3.5 times the peak area of astromicin from the standard solution.

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 340 nm; detection wavelength: 430 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A stainless steel tube 0.25 mm in inside diameter and 150 cm in length.

Temperature of reaction coil: 50°C

Mobile phase: To 800 mL of a solution of anhydrous sodium sulfate (71 in 2000) add 25 mL of a solution of sodium 1-heptanesulfonate (1 in 1000) and 1 mL of acetic acid (100), and add water to make 1000 mL.

Reaction reagent: Dissolve 11.2 g of potassium hydroxide, 0.458 g of polyoxyethylene (23) lauryl ether, 0.300 g of *o*-phthalaldehyde and 1 mL of 2-mercaptoethanol in 400 mL of a solution of boric acid (31 in 1000), and add water to make 500 mL.

Reaction temperature: 50°C

Flow rate of mobile phase: 0.7 mL per minute

Flow rate of reaction reagent: 0.2 mL per minute

Time span of measurement: About 2 times as long as the retention time of astromicin.

System suitability—

Test for required detectability: To 5 mL of the sample solution add water to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add water to make exactly 100 mL. Confirm that the peak area of astromicin obtained from 10 μL of this solution is equivalent to 1.5 to 2.5% of that from 10 μL of the solution for system suitability test.

System performance: To 100 mL of water add 5 mL of the sample solution and 2 mL of a solution of L-valine (1 in 5000). When the procedure is run with 10 μL of this solution under the above operating conditions, L-valine and astromicin are eluted in this order with the resolution between these peaks being not less than 1.5, and when the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the symmetry coefficient of the peak of astromicin is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of astromicin is not more than 2.0%.

Water Not more than 8.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Astromicin Sulfate Reference Standard, equivalent to about 25 mg (potency), dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0

to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Astromicin Sulfate, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Bacampicillin Hydrochloride

塩酸バカンピシリン

Change the origin/limits of content to read:

Bacampicillin Hydrochloride is a hydrochloride of ampicilline ethoxycarbonyloxyethyl ester.

Bacampicillin Hydrochloride contains not less than 626 μg (potency) per mg, calculated on the anhydrous basis. The potency of Bacampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Add the following next to Identification:

Optical rotation $[\alpha]_D^{20}$: +140 – +170° (0.1 g calculated on the anhydrous basis, ethanol (95), 25 mL, 100 mm).

Change the Purity to read:

Purity **(1)** Heavy metals—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Bacampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Bacampicillin Hydrochloride, transfer into a 100-mL separater, add exactly 15 mL of ice-cold water to dissolve, add and mix with exactly 10 mL of ice-cold 0.05 mol/L phosphate buffer solution, pH 7.0, then add 25 mL of ice-cold chloroform, shake, and abandon the chloroform layer. Repeat the procedure twice with two 25-mL portions of ice-cold chloroform. Centrifuge the water layer, filter the supernatant, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 20 mg, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0 and water to make exactly 25 mL, and use this solution as the standard solution. To exactly 10 mL each of the sample solution and the standard solution add exactly 2 mL of sodium hydroxide TS, allow to stand for exactly 15 minutes, add exactly 2 mL of 1 mol/L hydrochloric acid TS, exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6, and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 20 minutes without exposure to light. Titrate these solutions with 0.01 mol/L sodium thiosulfate VS until the color of the solution changes to colorless. Separately, to exactly 10 mL each of the sample solution and the standard solution add exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, and perform a blank determination with the same manner. Determine the consumed amounts (mL) of 0.005 mol/L iodine VS, V_T and V_S, of the sample solution and the standard solution: the amount of ampicillin is not more than 1.0%.

$$\text{Amount (mg) of ampicillin } (C_{16}H_{19}N_3O_4S) = W_S \times \frac{V_T}{V_S} \times \frac{1}{20}$$

W_S: Amount (mg) of Ampicillin Reference Standard

Change the Assay to read:

Assay Weigh accurately an amount of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride Reference Standard, equivalent to about 40 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the peak areas, A_T and A_S, of bacampicillin of these solutions.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Bacampicillin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of diluted 2 mol/L sodium dihydrogen phosphate TS (1 in 100), add diluted 0.05 mol/L disodium hydrogen phosphate TS (2 in 5) to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bacampicillin is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry constant of the peak of bacampicillin are not less than 10,000 and not more than 2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of bacampicillin is not more than 2.0%.

Add the following:

Bacitracin

バシトラシン

[*1405-87-4*]

Bacitracin is a mixture of peptide substances having antibacterial activity including bacitracin A as the main component produced by the growth of *Bacillus subtilis* or *Bacillus licheniformis*.

It contains not less than 40 Units per mg. The potency of Bacitracin is expressed as unit calculated from the amount of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$: 1422.69). 1 unit of Bacitracin is equivalent to 23.8 μg of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$).

Description Bacitracin occurs as a white to light brown powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** To 3 mL of a solution of Bacitracin (1 in 100) add 3 mL of 4-dimethylaminobenzaldehyde TS, shake until red-rosy to red-purple color appears, then add several drops of a solution of sodium nitrite (1 in 100), and shake: a green to dark green color is produced.

(2) Dissolve 60 mg each of Bacitracin and Bacitracin Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatograph. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (30:15:10:6:5) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 110°C for 5 minutes: the spots obtained from the sample solution and the standard solution show the same *R*f value.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Bacitracin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Dissolve 0.15 g of Bacitracin in 0.05 mol/L sulfuric acid TS to make 100 mL. To 2 mL of this solution add 0.05 mol/L sulfuric acid TS to make 10 mL, and determine the absorbances of this solution, A_T and A_F, at 252 nm and 290 nm as directed under the Ultraviolet-visible Spectrophotometry: A_F/A_T is not more than 0.20.

Loss on drying Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Micrococcus luteus* ATCC 10240.

(2) Culture medium—Use the medium iii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Bacitracin Reference Standard, equivalent to about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Bacitracin, equivalent about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Bekanamycin Sulfate

硫酸ベカナマイシン

Change to read except the structural formula and chemical name:

Bekanamycin Sulfate contains not less than 680 μg (potency) per mg, calculated on the dried basis. The potency of Bekanamycin Sulfate is expressed as mass (potency) of bekanamycin ($C_{18}H_{37}N_5O_{10}$: 483.51).

Description Bekanamycin Sulfate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 20 mg of Bekanamycin Sulfate in 2 mL of 1/15 mol/L phosphate buffer solution, pH 5.6, add 1 mL of ninhydrin TS, and boil: a blue-purple color

develops.

(2) Dissolve 30 mg each of Bekanamycin Sulfate and Bekanamycin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R*f value.

(3) To a solution of Bekanamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation $[\alpha]_D^{20}$: +102 – +116° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.50 g of Bekanamycin Sulfate in 10 mL of water is between 6.0 and 8.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Bekanamycin Sulfate in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Bekanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Bekanamycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 60 mg of Bekanamycin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 7.8 to 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Bekanamycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Bekanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Benzbromarone

ベンズブロマロン

Change the origin/limits of content to read:

Benzbromarone, when dried, contains not less than 98.5% and not more than 101.0% of $C_{17}H_{12}Br_2O_3$.

Change the Description to read:

Description Benzbromarone occurs as a white to light yellow, crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Benzbromarone in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzbromarone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Change the Purity to read:

Purity **(1)** Sulfate—Dissolve 1.0 g of Benzbromarone in 40 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 40 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(2) Soluble halides—Dissolve 0.5 g of Benzbromarone in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Proceed with this solution as directed under the Chloride Limit Test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL.

(3) Heavy metals—Proceed with 2.0 g of Benzbromarone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

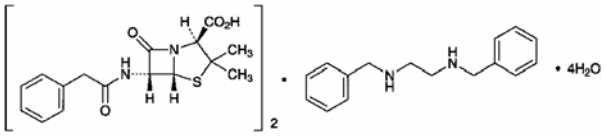
(4) Iron—Prepare the test solution with 1.0 g of Benzbromarone according to Method 3, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Benzbromarone in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 4-methyl-2-pentanone, ethanol (99.5) and acetic acid (100) (100:20:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Add the following:

Benzylpenicillin Benzathine

ベンジルペニシリンベンザチン



$(C_{16}H_{18}N_2O_4S)_2.C_{16}H_{20}N_2.4H_2O$: 981.18

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-phenylacetylamino-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid hemi(*N*,*N*′-dibenzylethylenediamine) dihydrate

[*41372-02-5*]

Benzylpenicillin Benzathine contains not less than 1152 units per mg, calculated on the anhydrous basis. The potency of Benzylpenicillin Benzathine is expressed as unit calculated from the amount of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$: 356.37). 1 unit of Benzylpenicillin Benzathine is equivalent to 0.6 μg of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$).

Description Benzylpenicillin Benzathine occurs as a white crystalline powder.

It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Benzylpenicillin Benzathine in methanol (1 in 2000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Benzathine as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +217 – +233° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Benzylpenicillin Benzathine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Benzylpenicillin Benzathine according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 70 mg of Benzylpenicillin Benzathine in 25 mL of methanol, add a solution prepared by dissolving 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and

use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 2.4 with respect to benzylpenicillin obtained from the sample solution is not more than 2 times the total area of the peaks of benzylpenicillin and benzathine obtained from the standard solution, and the total area of the peaks other than benzylpenicillin, benzathine and the peak having the relative retention time of about 2.4 obtained from the sample solution is not more than the total area of the peaks of benzylpenicillin and benzathine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (6:3:1).

Mobile phase B: A mixture of methanol, water and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (6:3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 10	75	25
10 – 20	75 → 0	25 → 100
20 – 55	0	100

Flow rate: 1.0 mL/min

Time span of measurement: About 3 times as long as the retention time of benzylpenicillin after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from the standard solution.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, benzathine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 2.0%.

Water 5.0 – 8.0% (1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium iii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Benzylpenicillin Sodium Reference Standard, equivalent to about 20,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Benzylpenicillin Benzathine, equivalent about 20,000 units, and dissolve in *N*,*N*-dimethylformamide to make exactly 10 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Benzylpenicillin Potassium

ベンジルペニシリンカリウム

Change to read except the structural formula and chemical name:

Benzylpenicillin Potassium contains not less than 1430 units per mg, calculated on the dried basis. The potency of Benzylpenicillin Potassium is expressed as mass unit of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$). One unit of Benzylpenicillin Potassium is equivalent to 0.57 μg of benzylpenicillin potassium.

Description Benzylpenicillin Potassium occurs as white, crystals or crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Benzylpenicillin Potassium (1 in 1000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Benzylpenicillin Potassium Reference Standard prepared in the same manner as the

sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Potassium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Benzylpenicillin Potassium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Benzylpenicillin Potassium responds to the Qualitative Test (1) for potassium salt.

Optical rotation $[\alpha]_D^{20}$: +270 – +300° (1.0 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Benzylpenicillin Potassium in 100 mL of water is between 5.0 and 7.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1 g of Benzylpenicillin Potassium in 10 mL of water is clear, and colorless or light yellow.

(2) Heavy metals—Proceed with 2.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution by incinerating 1.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. In the incineration, use a crucible of porcelain, and after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 1 mL of hydrogen peroxide (30), then burn the ethanol (not more than 2 ppm).

(4) Related substances—Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than benzylpenicillin obtained from the sample solution is not more than the peak area of benzylpenicillin from the standard solution, and the total area of the peaks other than benzylpenicillin from the sample solution is not more than 3 times the peak area of benzylpenicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (33 in 5000) and acetonitrile (19:6), adjusted the pH to 8.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 minutes.

Time span of measurement: About 5 times as long as the retention time of benzylpenicillin.

System suitability—

Test for required detection: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water. Separately, dissolve 10 mg of methyl parahydroxybenzoate in 20 mL of acetonitrile. To 1 mL of this solution add water to make 20 mL. Mix 1 mL each of these solutions, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, benzylpenicillin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 2.0%.

Loss on drying Not more than 1.0% (3 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium iii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Benzylpenicillin Potassium Reference Standard, equivalent to about 40,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Benzylpenicillin Potassium, equivalent to about 40,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Betamethasone Sodium Phosphate

リン酸ベタメタゾンナトリウム

Change the Assay to read:

Assay Weigh accurately about 20 mg each of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate Reference Standard (determine its water content before using in the same manner as Betamethasone Sodium Phosphate), and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, and exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{22}H_{28}FNa_2O_8P = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Betamethasone Sodium Phosphate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of tetra-*n*-butylammonium bromide, 3.2 g of disodium hydrogen phosphate 12-water and 6.9 g of potassium dihydrogen phosphate in 1000 mL of water, and add 1500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of betamethasone phosphate is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone phosphate to that of the internal standard is not more than 1.0%.

Betamethasone Valerate

吉草酸ベタメタゾン

Change the Assay to read:

Assay Dissolve about 10 mg each of Betamethasone Valerate and Betamethasone Valerate Reference Standard, previously dried and accurately weighed, in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone valerate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{27}H_{37}FO_6 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Betamethasone Valerate Reference Standard

Internal standard solution—A solution of isoamyl benzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

Bisacodyl Suppositories

ビサコジル坐剤

Change the Assay to read:

Assay Weigh accurately not less than 20 Bisacodyl Suppositories, make them fine fragments carefully, and mix uniformly. Weigh accurately a portion of the fragments, equivalent to about 10 mg of bisacodyl ($C_{22}H_{19}NO_4$), add 40 mL of tetrahydrofuran, warm to 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 100 mL. Cool this solution in ice for 30 minutes, centrifuge, filter the supernatant liquid through a membrane filter with pore size of 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Bisacodyl Reference Standard, previously dried at 105°C for 2 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bisacodyl to that of the internal standard, respectively.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Bisacodyl Reference Standard

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L citric acid TS, acetonitrile and methanol (2:1:1).

Flow rate: Adjust the flow rate so that the retention time of bisacodyl is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of bisacodyl to that of the internal standard is not more than 1.0%.

Bleomycin Hydrochloride

塩酸ブレオマイシン

Change to read except the structural formula and chemical name:

Bleomycin Hydrochloride contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg. The potency of Bleomycin Hydrochloride is expressed as mass (potency) of bleomycin A_2 ($C_{55}H_{84}ClN_{17}O_{21}S_3$: 1451.00).

Description Bleomycin Hydrochloride occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 4 mg of Bleomycin Hydrochloride add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Hydrochloride (1 in 100) responds to the Qualitative Test (2) for chloride.

pH The pH of a solution obtained by dissolving 0.10 g of Bleomycin Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Hydrochloride in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A_2 (the first principal peak) is between 55% and 70%, that of bleomycin B_2 (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A_2 and bleomycin B_2 is not less than 85%, the peak area of demethylbleomycin A_2 (a peak having the relative retention time of 1.5 – 2.5 to bleomycin A_2) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle

diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL per minute.

Time span of measurement: 20 minutes after elution of the peak of demethylbreomycin A_2 after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0%.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.08 g of Bleomycin Hydrochloride in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Hydrochloride in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Copper hollow-cathode lamp
Wavelength: 324.8 nm

Loss on drying Not more than 5.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Mycobacterium smegmatis* ATCC 607

(2) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(3) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(4) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(5) Preparation of cylinder-agar plate—Proceed as directed in the Preparation of cylinder-agar plate under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(6) Standard solutions—Weigh accurately an amount of Bleomycin A_2 Hydrochloride Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(7) Sample solutions—Weigh accurately an amount of Bleomycin Hydrochloride, equivalent to about 15 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions

as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Bleomycin Sulfate

硫酸ブレオマイシン

Change to read except the structural formula and chemical name:

Bleomycin Sulfate contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg. The potency of Bleomycin Sulfate is expressed as mass (potency) of bleomycin A_2 ($C_{55}H_{84}ClN_{17}O_{21}S_3$: 1451.00).

Description Bleomycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 4 mg of Bleomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Sulfate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Bleomycin Sulfate responds to the Qualitative Tests (1) and (2) for sulfate.

pH The pH of a solution obtained by dissolving 10 mg of Bleomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Sulfate in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A_2 (the first principal peak) is between 55% and 70%, that of bleomycin B_2 (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A_2 and bleomycin B_2 is not less than 85%, the peak area of demethylbleomycin A_2 (a peak having the relative retention time of 1.5 – 2.5 against bleomycin A_2) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL/min

Time span of measurement: Twenty minutes after elution of the peak of demethylbleomycin A_2 after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0%.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 0.08 g of Bleomycin Sulfate in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Sulfate in 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene
Supporting gas—Air

Lamp: Copper hollow-cathode lamp

Wavelength: 324.8 nm

Loss on drying Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be

tested while avoiding moisture absorption.

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Mycrobacterium smegmatis* ATCC 607

(2) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(3) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(4) Preparation of seeded agar layer – Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(5) Preparation of cylinder-agar plate—Proceed as directed in 7. Preparation of cylinder-agar plate under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(6) Standard solutions—Weigh accurately an amount of Bleomycin A_2 Hydrochloride Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(7) Sample solutions—Weigh accurately an amount of Bleomycin Sulfate, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Calcium Chloride Injection

塩化カルシウム注射液

Change the origin/limits of content to read:

Calcium Chloride Injection is an aqueous solution for injection. It contains not less than 95.0% and not more than 105.0% of the labeled amount of calcium chloride ($CaCl_2$: 110.98).

The concentration of Calcium Chloride Injection is expressed as the quantity of calcium chloride ($CaCl_2$).

Change the Description to read:

Description Calcium Chloride Injection is a clear, colorless liquid.

Add the following next to Identification:

pH 4.5 – 7.5

Bacterial endotoxins Less than 0.30 EU/mg.

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Calcium Polystyrene Sulfonate

ポリスチレンスルホン酸カルシウム

Change the Purity (4) to read:

Purity

(4) Styrene—To 10.0 g of Calcium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, dilute with acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions. Determine the peak heights, H_T and H_S, of styrene in each solution: H_T is not larger than H_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter

and 2 m in length, having polyethylene glycol 20 M coated at the ratio of 15% on siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of styrene is about 9 minutes.

System suitability—

System performance: Mix 10 mg of styrene with 1000 mL of acetone. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of styrene are not less than 800 and 0.8 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of styrene is not more than 5%.

d-Camphor

d-カンフル

Change the Purity (2) to read:

Purity

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *d*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

dl-Camphor

dl-カンフル

Change the Purity (2) to read:

Purity

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *dl*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

Carbazochrome Sodium Sulfonate

カルバゾクロムスルホン酸ナトリウム

Change the Purity (3) to read:

Purity

(3) Related substances—Dissolve 50 mg of Carbazochrome Sodium Sulfonate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of carbazochrome sulfonate from the sample solution is not larger than the peak area of carbazochrome sulfonate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.2 g of ammonium dihydrogen phosphate in 1000 mL of water, and filter through a membrane filter (0.4 μm in pore size) if necessary. To 925 mL of this solution add 75 mL of ethanol (95), shake, and adjust the pH to 3 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbazochrome sulfonate is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of carbazochrome sulfonate after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbazochrome sulfonate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of carbazochrome sulfonate obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg each of Carbazochrome Sodium Sulfonate and carbazochrome in 100 mL of water by warming. When the procedure is run with 10 μL of this solution under the above operating conditions, carbazochrome sulfonate and carbazochrome are elut-

ed in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbazochrome sulfonate is not more than 2.0%.

Carbidopa

カルビドパ

Change the Purity (2) to read:

Purity

(2) Related substances—Dissolve 50 mg of Carbidopa in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of all peaks other than the peak of carbidopa from the sample solution is not larger than the peak area of carbidopa from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carbidopa.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbidopa obtained from 20 μL of this solution is equivalent to 7 to 13% of that of carbidopa obtained from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Change the Assay to read:

Assay Weigh accurately about 50 mg each of Carbidopa and Carbidopa Reference Standard (determined separately the loss on drying), and dissolve each in 70 mL of the mobile phase, by warming and using ultrasonication if necessary. After cooling, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of carbidopa in each solution.

$$\text{Amount (mg) of } C_{10}H_{14}N_2O_4.H_2O = W_S \times \frac{A_T}{A_S} \times 1.080$$

W_S: Amount (mg) of Carbidopa Reference Standard, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 950 mL of 0.05 mol/L sodium dihydrogen phosphate TS add 50 mL of ethanol (95), and adjust the pH to 2.7 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbidopa is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg each of Carbidopa and methyldopa in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, methyldopa and carbidopa are eluted in this order with the resolution between these peaks being not less than 0.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbidopa is not more than 1.0%.

Carumonam Sodium

カルモナムナトリウム

Change to read except the structural formula and chemical name:

Carumonam Sodium contains not less than 850 μg (potency) per mg, calculated on the anhydrous basis. The potency of Carumonam Sodium is expressed as mass (potency) of carumonam ($C_{12}H_{14}N_6O_{10}S_2$: 466.40).

Description Carumonam Sodium occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in formamide, very slightly soluble in methanol, and practically insoluble in acetic acid (100) and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Carumonam Sodium (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carumonam Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carumonam Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Carumonam Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Carumonam Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ5.5 ppm, and a single signal B at around δ7.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Carumonam Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +18.5 – +21.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Carumonam Sodium in 10 mL of water is between 5.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Carumonam Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 2.0 g of Carumonam Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Carumonam Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substance 1—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 4.0%, and each amount of the related substances other than the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 1.0%.

$$\text{Amount (\%) of related substance} = \frac{W_S}{W_T} \times \frac{A_T}{A_S}$$

W_S: Amount (g) of Carumonam Sodium Reference Standard

W_T: Amount (g) of the sample

A_S: Peak area of carumonam from the standard solution

A_T: Each peak area other than carumonam from the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carumonam.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.9%.

(5) Related substance 2—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of each related substance is not more than 1.0%.

$$\text{Amount (\%) of related substance} = \frac{W_S}{W_T} \times \frac{A_T}{A_S}$$

W_S: Amount (g) of Carumonam Sodium Reference Standard

W_T: Amount (g) of the sample

A_S: Peak area of carumonam from the standard solution

A_T: Each area of the peaks appeared after the peak of carumonam from the sample solution

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (74:25:1).

Flow rate: Dissolve 0.01 g of phthalic acid in the mobile phase to make 100 mL. Adjust the flow rate so that the retention time of phthalic acid is about 6.5 minutes when the procedure is run with 10 μL of this solution.

Time span of measurement: About 10 times as long as the retention time of carumonam.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.9%.

(6) Total amount of related substances − The total of the amounts of the related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.0%.

Water Not more than 2.0% (0.2 g, volumetric titration, direct titration; Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Carumonam Sodium and Carumonam Sodium Reference Standard, equivalent to about 40 mg (potency), and dissolve each in the mobile phase to make exactly 20 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of carumonam to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of carumonam } (C_{12}H_{14}N_6O_{10}S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Carumonam Sodium Reference Standard

Internal standard solution—A solution of resorcinol in the mobile phase (9 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of carumonam is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of carumonam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefaclor

セファクロル

Change to read except the structural formula and chemical name:

Cefaclor contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefaclor is expressed as mass (potency) of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

Description Cefaclor occurs as a white to yellowish white crystalline powder.

It is slightly soluble in water and in methanol, and practically insoluble in ethanol (99.5) and in *N*,*N*-dimethylformamide.

Identification **(1)** Determine the absorption spectrum of a solution of Cefaclor (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefaclor as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.04 g of Cefaclor in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy and 1 drop of deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits an AB type quartet signal A at around δ3.7 ppm, and a single signal or a sharp multiple signal B at around δ7.6 ppm. The ratio of the integrated intensity of each signal, A:B, is about 2:5.

(4) Perform the test with Cefaclor as directed under the Flame Coloration Test (2): a green color appears.

Optical rotation $[\alpha]_D^{20}$: +105 – +120° (0.1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Cefaclor according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution by suspending 1.0 g of Cefaclor in 10 mL of *N*,*N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Cefaclor in 10 mL of sodium dihydrogen phosphate TS, pH 2.5, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas other than cefaclor from the sample solution are not more than 1/2 of the peak area of cefaclor from the standard solution, and the total of the peak areas other than cefaclor from the sample solution is not more than 2 times of the peak area of cefaclor from the standard solution. If necessary, proceed with 20 μL of sodium dihydrogen phosphate TS, pH 2.5 in the same manner as above to compensate the base line.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: To 550 mL of the mobile phase A add 450 mL of acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 30	95 → 75	5 → 25
30 – 45	75 → 0	25 → 100
45 – 55	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefaclor after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 20 μL of this solution is equivalent to 4 to 6% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of cefaclor are not less than 40,000 steps and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas and the retention times of cefaclor are not more than 2.0%, respectively.

Water Not more than 6.5% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefaclor and Cefaclor Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefaclor to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{14}ClN_3O_4S = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle di-

ameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.4 with diluted phosphoric acid (3 in 500). To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefaclor is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefaclor and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefaclor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefaloridin

セファロリジン

Change to read except the structural formula and chemical name:

Cefaloridin contains not less than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefaloridin is expressed as mass (potency) of cefaloridin ($C_{19}H_{17}N_3O_4S_2$).

Description Cefaloridin occurs as white to light yellowish white, crystals or crystalline powder.

It is soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefaloridin (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefaloridin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefaloridin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefaloridin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefaloridin in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ3.8 ppm, and two multiple signals, B and C, between δ6.9 ppm and δ7.5 ppm and between δ7.9 ppm and δ9.0 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 2:3:5.

Optical rotation $[\alpha]_D^{20}$: +46 – +51° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

pH Dissolve 1.0 g of Cefaloridin in 10 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution – Dissolve 1.0 g of Cefaloridin in 5 mL of water by warming: the solution is clear and light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefaloridin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefaloridin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 20 mg of Cefaloridin in 20 mL of a solution of sodium dihydrogen phosphate dihydrate (39 in 1250). To 2 mL of this solution add a solution of sodium dihydrogen phosphate dihydrate (39 in 1250) to make 20 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of each peak other than cefaloridin is not more than 0.5%, and the total amount of the peaks other than cefaloridin is not more than 2.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 25.9 g of sodium acetate trihydrate add 0.6 mL of acetic acid (100) and water to make 1000 mL. To 900 mL of this solution add 50 mL of acetonitrile and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefaloridin is about 14 minutes.

Time span of measurement: About 2 times as long as the retention time of cefaloridin after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, and add a solution of sodium dihydrogen phosphate dihydrate (39 in 1250) to make exactly 100 mL. To exactly 1 mL of this solution add a solution of sodium dihydrogen phosphate dihydrate (39 in 1250) to

make exactly 10 mL. Confirm that the peak area of cefaloridin obtained from 20 μL of this solution is equivalent to 0.07 to 0.13% of that from 20 μL of the sample solution.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of cefaloridin are not less than 5000 steps and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of cefaloridin is not more than 3.0%.

Water Not more than 4.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefaloridin and Cefaloridin Reference Standard, equivalent to about 45 mg (potency), and dissolve each in water to make exactly 50 mL. Measure exactly 20 mL each of these solutions, add exactly 5 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefaloridin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{19}H_{17}N_3O_4S_2 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefaloridin Reference Standard

Internal standard solution—A solution of acetanilide in acetonitrile (9 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 25.9 g of sodium acetate trihydrate in 0.6 mL of acetic acid (100) and water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefaloridin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefaloridin and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefaloridin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefalotin Sodium

セファロチンナトリウム

Change to read except the structural formula and chemical name:

Cefalotin Sodium contains not less than 910 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalotin Sodium is expressed as mass (potency) of cefalotin ($C_{16}H_{16}N_2O_6S_2$: 396.44).

Description Cefalotin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Cefalotin Sodium (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefalotin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalotin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefalotin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefalotin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ2.1 ppm, a single or sharp multiple signal B at around δ3.9 ppm, and a multiple signal C at around δ7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:2.

(4) Cefalotin Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{25}$: +124 – +134° (5 g, water, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Cefalotin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefalotin Sodium in 5 mL of water: the solution is clear

and light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefalotin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefalotin Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Pipet 1 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution obtained in the Assay and the standard solution prepared here as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefalotin from the sample solution is not more than the peak area of cefalotin from the standard solution, and the total area of the peaks other than cefalotin from the sample solution is not more than 3 times the peak area of cefalotin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefalotin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefalotin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin, is not less than 9, and the symmetry coefficient of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 2.0%.

Water Not more than 1.0% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefalotin Sodium and Cefalotin Sodium Reference Standard, equivalent to about 25 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefalotin.

Amount [μg (potency)] of cefalotin ($C_{16}H_{16}N_2O_6S_2$)

$$= W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefalotin Sodium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17 g of sodium acetate trihydrate in 790 mL of water, and add 0.6 mL of acetic acid (100). If necessary adjust the pH to 5.9 ± 0.1 with 0.1 mol/L sodium hydrochloride TS or acetic acid (100). To this solution add 150 mL of acetonitrile and 70 mL of ethanol (95).

Flow rate: Adjust the flow rate so that the retention time of cefalotin is about 12 minutes.

System suitability—

System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin is not less than 9, and the symmetry coefficient of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefamandole Sodium

セファマンドールナトリウム

Change to read except the structural formula and chemical name:

Cefamandole Sodium contains not less than 858 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefamandole Sodium is expressed as mass (potency) of cefamandole ($C_{18}H_{18}N_6O_5S_2$: 462.50).

Description Cefamandole Sodium occurs as a white to light yellowish white crystalline powder.

It is very soluble in water, freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefamandole Sodium (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefamandole Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefamandole Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ4.0 ppm and at around δ5.3 ppm, and a multiple signal C at around δ7.5 ppm. The ratio of the integrated intensity of the two signals, B:C, is about 1:5.

(4) Cefamandole Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: -26.0 – $-34.0°$ (1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Cefamandole Sodium in 10 mL of water is between 4.3 and 6.3.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Cefamandole Sodium in 2 mL of water: the solution is clear and pale yellow to light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefamandole Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefamandole Sodium according to Method 5, and perform the test (not more than 2 ppm).

(4) 1-Methyl-1*H*-tetrazole-5-thiol—Dissolve 30 mg of Cefamandole Sodium in exactly 2 mL of the internal standard solution, add water to make 20 mL, and use this solution as the sample solution. Separately, dissolve 9 mg of sodium 1-methyl-1*H*-tetrazole-5-thiolate in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of 1-methyl-1*H*-tetrazole-5-thiol to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of *m*-cresol (7 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate 12-water and 1.60 g of tetra-*n*-butylammonium bromide in water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of 1-methyl-1*H*-tetrazole-5-thiol is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, 1-methyl-1*H*-tetrazole-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 14.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 1-methyl-1*H*-tetrazole-5-thiol to that of the internal standard is not more than 3.0%.

Water Not more than 2.0% (1 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefamandole Sodium and Cefamandole Lithium Reference Standard, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 100 mL. Measure exactly 2 mL each of these solutions, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefamandole to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefamandole } (C_{18}H_{18}N_6O_5S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefamandole Lithium Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with divinylbenzene-methacrylate co-polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

25°C.

Mobile phase: Dissolve 15.0 g of disodium hydrogen phosphate 12-water and 1.01 g of potassium dihydrogen phosphate in water to make 1000 mL, and add 1.6 g of tetra-*n*-butylammonium bromide. To 810 mL of this solution add 290 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefamandole is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, cefamandole and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefamandole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefbuperazone Sodium

セフブペラゾンナトリウム

Change to read except the structural formula and chemical name:

Cefbuperazone Sodium contains not less than 870 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefbuperazone Sodium is expressed as mass (potency) of cefbuperazone ($C_{22}H_{29}N_9O_9S_2$: 627.65).

Description Cefbuperazone Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol and in pyridine, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Cefbuperazone Sodium (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g of Cefbuperazone Sodium in 0.5 mL of deuterated pyridine for nuclear magnetic resonance spectroscopy and 1 drop of heavy water for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ1.1 ppm, and two doublet signals, B and C, at around δ1.6 ppm and at around δ5.1 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefbuperazone Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +48 – +56° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the solution is clear and light yellow.

(2) Heavy metals—Proceed with 2.0 g of Cefbuperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefbuperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefbuperazone Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the percentages of each peak area of related substances from the sample solution against 50 times of the peak area of cefbuperazone from the standard solution; the amount of related substance I having the relative retention time of about 0.2 to cefbuperazone is not more than 2.0%, the amount of related substance II having the relative retention time of about 0.6 to cefbuperazone is not more than 4.5% and the amount of related substance III having the relative retention time of about 1.6 to cefbuperazone is not more than 1.0%, and the total amount of these related substances is not more than 6.0%. For these calculations, use the values of the peak areas of the related substances I and III obtained by the automatic integration method after multiplying by each sensitivity coefficient, 0.72 and 0.69, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefbuperazone.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefbuperazone obtained from 25 μL of this solution is equivalent to 7 to 13% of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of cefbuperazone are not less than

5000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefbuperazone is not more than 2.0%.

Water Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefbuperazone Sodium and Cefbuperazone Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL. Measure exactly 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefbuperazone to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefbuperazone } (C_{22}H_{29}N_9O_9S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefbuperazone Reference Standard

Internal standard solution—A solution of acetonitrile in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.0 g of tetra-*n*-propylammonium bromide in 1000 mL of a mixture of water, acetonitrile and acetic acid-sodium acetate buffer solution, pH 5.0 (83:13:4).

Flow rate: Adjust the flow rate so that the retention time of cefbuperazone is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefbuperazone are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefbuperazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Cefmenoxime Hydrochloride

塩酸セフメノキシム

Change to read except the structural formula and chemical name:

Cefmenoxime Hydrochloride contains not less than 890 μg (potency) per mg, calculated on the dehydrated basis. The potency of Cefmenoxime Hydrochloride is expressed as mass (potency) of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$: 511.57).

Description Cefmenoxime Hydrochloride occurs as white to light orange-yellow crystals or crystalline powder.

It is freely soluble in formamide and in dimethylsulfoxide, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution, pH 6.8 (3 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefmenoxime Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmenoxime Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefmenoxime Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefmenoxime Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ3.9 ppm, and a single signal C at around δ6.8 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Dissolve 0.01 g of Cefmenoxime Hydrochloride in 1 mL of diluted sodium carbonate TS (1 in 20), add 5 mL of acetic acid (100) and 2 drops of silver nitrate TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: -27 – $-35°$ (1 g, 0.1 mol/L phosphate buffer solution, pH 6.8, 100 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Cefmenoxime Hydrochloride in 150 mL of water is between 2.8 and 3.3.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefmenoxime Hydrochloride in 10 mL of diluted sodium carbonate TS (1 in 4) is clear and colorless to light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution by incinerating 1.0 g of Cefmenoxime Hydrochloride according to Method 4 and adding 10 mL of dilute hydrochloric acid to the residue after cooling, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 50 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 1-methyl-1*H*-tetrazol-5-thiol, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (1). Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride Reference Standard, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (2). Perform the test immediately after preparation of these solutions with exactly 10 μL each of the sample solution, the standard solution (1) and the standard solution (2) as directed under the Liquid Chromatography according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method, and calculate the amounts of 1-methyl-1*H*-tetrazol-5-thiol and the total related substance by the following formula: the amount of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%, and the total related substance is not more than 3.0%.

$$\text{Amount (\%) of 1-methyl-1}H\text{-tetrazol-5-thiol} = \frac{W_{Sa}}{W_T} \times \frac{A_{Ta}}{A_{Sa}} \times 20$$

$$\text{Amount (\%) of total related substance} = \frac{W_{Sa}}{W_T} \times \frac{A_{Ta}}{A_{Sa}} \times 20 + \frac{W_{Sb}}{W_T} + \frac{S_T}{A_{Sb}} \times 5$$

W_{Sa}: Amount (g) of 1-methyl-1*H*-tetrazol-5-thiol
W_{Sb}: Amount (g) of Cefmenoxime Hydrochloride Reference Standard
W_T: Amount (g) of the sample
A_{Sa}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the standard solution (1)
A_{Sb}: Peak area of cefmenoxime from the standard solution (2)
A_{Ta}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the sample solution
S_T: Total area of the peaks other than 1-methyl-1*H*-tetrazol-5-thiol and other than cefmenoxime from the sample solution

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of cefmenoxime.

System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution (1), add the mobile phase to make exactly 100 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazol-5-thiol obtained from 10 μL of this solution is equivalent to 4.5 to 5.5% of that from the standard solution (1). Then, measure exactly 2 mL of the standard solution (2), add the mobile phase to make exactly 100 mL. Confirm that the peak area of cefmenoxime obtained from 10 μL of this solution is equivalent to 1.5 to 2.5% of that from the standard solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%.

Water Not more than 1.5% (1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (2:1)).

Assay Weigh accurately an amount of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in 10 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, add exactly 20 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefmenoxime to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefmenoxime } (C_{16}H_{17}N_9O_5S_3) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefmenoxime Hydrochloride Reference Standard

Internal standard solution—A solution of phthalimide in methanol (3 in 2000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:10:1).

Flow rate: Adjust the flow rate so that the retention time of cefmenoxime is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmenoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.3.

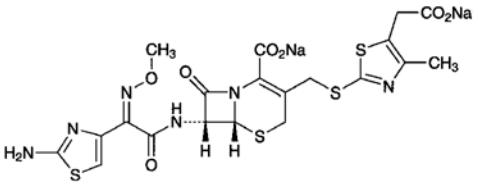
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefmenoxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Add the following:

Cefodizime Sodium

セフォジジムナトリウム



$C_{20}H_{18}N_6Na_2O_7S_4$: 628.63

Disodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-[(5-carboxylatomethyl-4-methylthiazol-2-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicycle[4.2.0]oct-2-ene-2-carboxylate [*86329-79-5*]

Cefodizime Sodium contains not less than 890 μg (potency) per mg, calculated on the anhydrous basis and corrected by the ethanol amount. The potency of Cefodizime Sodium is expressed as mass (potency) of cefodizime ($C_{20}H_{20}N_6O_7S_4$: 584.67).

Description Cefodizime Sodium occurs as a white to light yellowish white crystalline powder.

It is very soluble in water, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefodizime Sodium (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefodizime Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefodizime Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefodizime Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefodizime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ2.3 ppm, at around δ4.0 ppm, and at around δ7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:3:1.

(4) Cefodizime Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: −56 – −62° (0.2 g calculated on the anhydrous basis and corrected by the ethanol amount, water, 20 mL, 100 mm).

pH Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the solution is clear and pale yellow to light yellow.

(2) Heavy metals—Weigh 1.0 g of Cefodizime Sodium in a crucible, cover loosely, and carbonize by gentle heating. After cooling, add 2 mL of sulfuric acid, heat gradually until the white fumes are no longer evolved, and ignite between 500°C and 600°C. Proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefodizime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 30 mg of Cefodizime Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefodizime from the sample solution is not more than the peak area of cefodizime from the standard solution, and the total area of the peaks other than cefodizime from the sample solution is not more than 3 times the peak area of cefodizime from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefodizime after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefodizime obtained from 5 μL of this solution is equivalent to 7 to 13%

of that from 5 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(5) Ethanol—Weigh accurately about 1 g of Cefodizime Sodium, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 2 g of ethanol for gas chromatography, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard: the amount of ethanol is not more than 2.0%.

$$\text{Amount (\%) of ethanol} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S}$$

W_S: Amount (g) of ethanol for gas chromatography
W_T: Amount (g) of the sample

Internal standard solution—A solution of 1-propanol (1 in 400).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 3 m in length, packed with tetrafluoroethylene polymer for gas chromatography (180 – 250 μm in particle diameter) coated in 15% with polyethylene glycol 20 M.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefodizime Sodium and Cefodizime Sodium Reference Standard, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add water to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefodizime to that of the internal standard.

$$\text{Amount [μg (potency)] of cefodizime } (C_{20}H_{20}N_6O_7S_4) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefodizime Sodium Reference Standard

Internal standard solution—A solution of anhydrous caffeine (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.80 g of potassium dihydrogen phosphate and 0.20 g of anhydrous disodium hydrogen phosphate in a suitable amount of water, and add 80 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefodizime is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefodizime and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefodizime to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefotaxime Sodium

セフォタキシムナトリウム

Change to read except the structural formula and chemical name:

Cefotaxime Sodium contains not less than 916 μg (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Sodium is expressed as mass (potency) of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47).

Description Cefotaxime Sodium occurs as white to light yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Dissolve 2 mg of Cefotaxime Sodium in 0.01 mol/L hydrochloric acid TS to make 100 mL. Deter-

mine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotaxime Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefotaxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three single signals, A, B and C, at around δ2.1 ppm, at around δ4.0 ppm and at around δ7.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefotaxime Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +58 – +64° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is clear and light yellow.

(2) Sulfate—Dissolve 2.0 g of Cefotaxime Sodium in 40 mL of water, add 2 mL of dilute hydrochloric acid and water to make 50 mL, shake well, and filter. Discard first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals—Proceed with 1.0 g of Cefotaxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic—Prepare the test solution with 1.0 g of Cefotaxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions, and determine each peak area obtained from the chromatogram by the automatic integration method: the each peak area other than cefotaxime is not more than 1.0% and the total of these peak areas is not more than 3.0%.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of cefotaxime after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of cefotaxime obtained from 10 μL of this solution is equivalent to 0.15 to 0.25% of that obtained from 10 μL of the standard solution.

Loss on drying Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately an amount of Cefotaxime Sodium and Cefotaxime Reference Standard, equivalent to about 40 mg (potency), dissolve each in the mobile phase A to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of cefotaxime of these solutions.

$$\text{Amount [}\mu\text{g (potency)] of cefotaxime } (C_{16}H_{17}N_5O_7S_2) = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefotaxime Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 860 mL of this solution add 140 mL of methanol.

Mobile phase B: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 600 mL of this solution add 400 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 7	100	0
7 – 9	100 → 80	0 → 20
9 – 16	80	20
16 – 45	80 → 0	20 → 100
45 – 50	0	100

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 14 minutes (about 1.3 mL/min).

System suitability—

System performance: To 1 mL of the standard solution add 7.0 mL of water and 2.0 mL of methanol, mix, then add 25 mg of sodium carbonate decahydrate, and shake. After allowing to stand for 10 minutes, add 3 drops of acetic acid (100) and 1 mL of the standard solution, and mix. When the procedure is run with 10 μL of this solution under the above operating conditions, desacetyl cefotaxime with the relative retention time being about 0.3 to cefotaxime and cefotaxime are eluted in this order with the resolution between these peaks being not less than 20, and the symmetry coefficient of the peak of cefotaxime is not more than 2.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotaxime is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefotetan

セフォテタン

Change to read except the structural formula and chemical name:

Cefotetan contains not less than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotetan is expressed as mass (potency) of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

Description Cefotetan occurs as white to light yellowish white powder.

It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Cefotetan in phosphate buffer solution for antibiotics, pH 6.5 (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotetan Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotetan as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotetan Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve 50 mg of Cefotetan in 0.5 mL of a solution of sodium hydrogen carbonate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25). Determine the spectrum of this solution as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C and D, at around δ 3.6 ppm, at around δ4.0 ppm, at around δ5.1 ppm and at around δ5.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C:D, is about 3:3:1:1.

Optical rotation $[\alpha]_D^{20}$: +112 – +124° (0.5 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 200), 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefotetan in 10 mL of a solution of sodium hydrogen carbonate (1 in 30): the solution is clear, and colorless or light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefotetan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Cefotetan, dissolve in a suitable amount of methanol, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and about 2 mg of Cefotetan Reference Standard, calculated on the anhydrous basis, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_{Ta}, Q_{Tb}, Q_{Tc}, Q_{Td}, Q_{Te} and Q_{Tf}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone having the relative retention time of about 0.5 with respect to cefotetan, Δ_2-cefotetan having the relative retention time of about 1.2 with respect to cefotetan, isothiazole substance having the relative retention time of about 1.3 with respect to cefotetan, each of other related substances and the total of other related substances, to the peak area of the internal standard, respectively, obtained from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol and cefotetan, to the peak area of the internal standard, respectively, obtained from the standard solution. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone, Δ_2-cefotetan, isothiazole substance, each of other related substances and the total of other related substances from the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol is not more than 0.3%, cefotetan lactone is not more than 0.3%, Δ_2-cefotetan is not more than 0.5%, isothiazole substance is not more than 0.5%, each of other related substances is not more than 0.2% and the total of other related substances is not more than 0.4%.

$$\text{1-Methyl-1}H\text{-tetrazole-5-thiol (\%)} = \frac{W_{Sa}}{W_T} \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{100}$$

$$\text{Cefotetan lactone (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{100}$$

$$\Delta_2\text{-Cefotetan (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tc}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Isothiazole substance (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Td}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Each of other related substances (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Te}}{Q_{Sb}} \times \frac{1}{100}$$

$$\text{Total of other related substances (\%)} = \frac{W_{Sb}}{W_T} \times \frac{Q_{Tf}}{Q_{Sb}} \times \frac{1}{100}$$

W_{Sa}: Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol
W_{Sb}: Amount (mg) of Cefotetan Reference Standard, calculated on the anhydrous basis
W_T: Amount (g) of the sample

Internal standard solution—A solution of anhydrous caffeine in methanol (3 in 10,000).
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 3.5 times as long as the retention time of cefotetan.
System suitability—
Test for required detectability: Measure exactly 15 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of cefotetan obtained from 5 μL of this solution is equivalent to 12 to 18% of that from 5 μL of the standard solution.
System performance: Proceed as directed in the system suitability in the Assay.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 2.0%.

Water Not more than 2.5% (1 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Isomer ratio Dissolve 10 mg of Cefotetan in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the adjacent two peaks appeared at around the retention time of 40 minutes, one having shorter retention time is *l*-substance and another having longer retention time is *d*-substance, by the area percentage method: the amount of *l*-substance is not less than 35% and not more than 45%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, water and a solution of tetrabutylammonium hydrogensulfate in acetonitrile (1 in 150) (9:9:2).
Flow rate: Adjust the flow rate so that the retention time of *l*-substance is about 40 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, *l*-substance and *d*-substance are eluted in this order with the resolution between these peaks being not less than 1.5.
System repeatability: To exactly 1 mL of the sample solution add methanol to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of *l*-substance is not more than 5%.

Assay Weigh accurately an amount of Cefotetan and Cefotetan Reference Standard, equivalent to about 50 mg (potency), and dissolve each in phosphate buffer solution for antibiotics, pH 6.5 to make exactly 50 mL. Pipet 15 mL each of these solutions, add exactly 10 mL of the internal standard solution and phosphate buffer solution for antibiotics, pH 6.5 to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefotetan to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{17}H_{17}N_7O_8S_4 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefotetan Reference Standard
Internal standard solution—A solution of anhydrous caffeine (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 11.53 g of phosphoric acid in 1000 mL of water. To 850 mL of this solution add 50 mL of acetonitrile, 50 mL of acetic acid (100) and 50 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of cefotetan is about 17 minutes.
System suitability—
System performance: When the procedure is run with 5 μL

of the standard solution under the above operating conditions, the internal standard and cefotetan are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefotiam Hexetil Hydrochloride

塩酸セフォチアムヘキセチル

Change to read except the structural formula and chemical name:

Cefotiam Hexetil Hydrochloride contains not less than 615 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hexetil Hydrochloride is expressed as mass (potency) of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Description Cefotiam Hexetil Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, in methanol and in ethanol (95), freely soluble in dimethylsulfoxide, and slightly soluble in acetonitrile.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefotiam Hexetil Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 125,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hexetil Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefotiam Hexetil Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ2.8 ppm and at around δ6.6 ppm, and a multiple signal, C, at around δ6.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 6:1:1.

(3) To a solution of Cefotiam Hexetil Hydrochloride (1 in 200) add 2 mL of dilute nitric acid and 1 mL of silver nitrate TS, and mix: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: +52 – +60° (0.1 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 3, and perform the test, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 1 ppm).

(3) Related substance 1—Weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride Reference Standard, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, is not more than 2.0%, and each amount of the other related substances is not more than 0.5%. For this calculation, use the value of the peak area obtained by the automatic integration method of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, after multiplying by its sensitivity coefficient, 0.78.

$$\text{Amount (\%) of each related substance} = \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times 5$$

W_S: Amount (g) of Cefotiam Hexetil Hydrochloride Reference Standard

W_T: Amount (g) of the sample

A_S: Total of two peak areas of cefotiam hexetil from the standard solution

A_T: Each peak area of related substance from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted 0.2 mol/L potassi-

um dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Mobile phase B: A mixture of acetonitrile, diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2) and acetic acid (100) (60:40:1).

Flowing of the mobile phase: Adjust so that the mixing rate of the mobile phase A and the mobile phase B is changed lineally from 1:0 to 0:1 for 30 minutes.

Flow rate: 0.7 mL per minute.

Time span of measurement: As long as about 3 times of the retention time of one of the cefotiam hexetil peaks, which appears first, after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Confirm that each area of the two peaks of cefotiam hexetil obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefotiam hexetil is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the total of the two peak areas of cefotiam hexetil is not more than 2.0%.

(4) Related substance 2—Weigh accurately about 20 mg of Cefotiam Hexetil Hydrochloride, dissolve in exactly 2 mL of methanol, add a mixture of a solution of diammonium hydrogen phosphate (79 in 20,000) and acetic acid (100) (200:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cefotiam Hydrochloride Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amounts of the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam are not more than 1.0%, respectively, and each amount of the related substances other than the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam is not more than 0.5%. For this calculation, use the value of the peak area of the related substance having the relative retention time of about 0.9 to cefotiam after multiplying by its sensitivity coefficient, 0.76.

$$\text{Amount (\%) of each related substance} = \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times 4$$

W_S: Amount (g) of Cefotiam Hydrochloride Reference Standard

W_T: Amount (g) of the sample

A_S: Peak area of cefotiam from the standard solution

A_T: Each peak area from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (79 in 20,000), methanol and acetic acid (100) (200:10:3).

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 15 minutes.

Time span of measurement: As long as about 2 times of the retention time of cefotiam after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of cefotiam obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that from 10 μL of the standard solution.

System performance: To 1 mL of a solution of acetaminophen in the mobile phase (1 in 50,000) add 3 mL of the standard solution, and mix well. When the procedure is run with 10 μL of this solution under the above operating conditions, acetaminophen and cefotiam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotiam is not more than 2.0%.

(5) Total amount of related substances—The total of the amount of related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.5%.

Water Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Isomer ratio Proceed the test with 20 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the conditions directed in the Assay, and determine the areas of the two peaks, A_a for the faster peak and A_b for the later peak, closely appeared each other at the retention time of around 10 minutes: $A_a/(A_a + A_b)$ is not less than 0.45 and not more than 0.55.

Assay Weigh accurately an amount of Cefotiam Hexetil Hydrochloride and Cefotiam Hexetil Hydrochloride Reference Standard, equivalent to about 30 mg (potency), and dissolve each in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly

50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefotiam hexetil to that of the internal standard. For this calculation, the total of the areas of the two peaks appeared closely each other at the retention time of around 10 minutes is used as the peak area of cefotiam hexetil.

$$\text{Amount [}\mu\text{g (potency)] of cefotiam } (C_{18}H_{23}N_9O_4S_3) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefotiam Hexetil Hydrochloride Reference Standard

Internal standard solution—A solution of benzoic acid in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) (7 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Flow rate: Adjust the flow rate so that the retention time of the faster peak of cefotiam hexetil is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and cefotiam hexetil are eluted in this order with the resolution between the two peaks of cefotiam hexetil being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefotiam hexetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefoxitin Sodium

セフォキシチンナトリウム

Change to read except the structural formula and chemical name:

Cefoxitin Sodium contains not less than 927 μg (potency) and not more than 970 μg (potency) per mg, calculated on the dehydrated, de-acetone and de-methanol basis. The potency of Cefoxitin Sodium is expressed as mass (potency) of cefoxitin ($C_{16}H_{17}N_3O_7S_2$: 427.45).

Description Cefoxitin Sodium occurs as white to light yellowish white granules or powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefoxitin Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 40,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefoxitin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ3.5 ppm, at around δ3.9 ppm and at around δ5.1 ppm, respectively, and a multiple signal D between δ6.9 ppm and δ7.5 ppm. The ratio of the integrated intensity of each signal, A:B:C:D, is about 3:2:1:3.

(3) A solution of Cefoxitin Sodium (1 in 10) responds to the Qualitative Tests for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +206 – +214° (0.25 g calculated on the dehydrated, de-acetone and de-methanol basis, methanol, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Cefoxitin Sodium in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefoxitin Sodium in 10 mL of water is clear and a pale yellow to yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefoxitin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cefoxitin Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Acetone and methanol—Dissolve 5.0 g of Cefoxitin Sodium in water to make exactly 50 mL. Pipet 3 mL of this solution in a centrifuge tube, allow to stand in an ice-cold water for 2 minutes, and add exactly 3 mL of diluted

hydrochloric acid (1 in 50). After mixing, centrifuge, and use the supernatant liquid as the sample solution. Separately, to exactly 5 mL of acetone add water to make exactly 1000 mL, and use this solution as the standard stock solution (1). Separately, to exactly 5 mL of methanol add water to make exactly 1000 mL, and use this solution as the standard stock solution (2). To exactly 50 mL of the standard stock solution (1) and exactly 5 mL of the standard stock solution (2) add water to make exactly 500 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and determine the peak areas of acetone, A_{Ta} and A_{Sa}, and the peak areas of methanol, A_{Tb} and A_{Sb}, of these solutions: the amounts of acetone and methanol are not more than 0.7% and not more than 0.1%, respectively.

$$\text{Amount (\%) of acetone} = \frac{A_{Ta}}{A_{Sa}} \times 0.791$$

$$\text{Amount (\%) of methanol} = \frac{A_{Tb}}{A_{Sb}} \times 0.0791$$

Operating conditions—

Detector: An hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (150 – 180 μm in particle diameter, 0.0085 μm in average pore size, 300 – 400 m^2/g).

Column temperature: A constant temperature of about 110°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acetone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, methanol and acetone are eluted in this order, and the number of theoretical plates of these peaks are not less than 160, respectively, and the symmetry constant of these peaks are not more than 2.3 and not more than 1.3, respectively.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acetone is not more than 5%.

(5) Lactone substance—Dissolve 0.25 g of Cefoxitin Sodium in 10 mL of water, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, acetone, water and acetic acid (100) (5:2:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot from the sample solution is not more intense than the principal spot from the standard solution.

Water Not more than 1.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefoxitin Sodium and Cefoxitin Reference Standard, equivalent to about 0.2 g (potency), and dissolve each in phosphate buffer solution, pH 6.0 to make exactly 100 mL. Pipet 15 mL each of these solutions, add exactly 5 mL of the internal standard solution and phosphate buffer solution, pH 6.0 to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefoxitin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefoxitin } (C_{16}H_{17}N_3O_7S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefoxitin Reference Standard

Internal standard solution—A solution of anhydrous caffeine in phosphate buffer solution, pH 6.0 (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (80:19:1).

Flow rate: Adjust the flow rate so that the retention time of cefoxitin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefoxitin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefoxitin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefpiramide Sodium

セフピラミドナトリウム

Change to read except the structural formula and chemical name:

Cefpiramide Sodium contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpiramide Sodium is expressed as mass (potency) of cefpiramide ($C_{25}H_{24}N_8O_7S_2$: 612.64).

Description Cefpiramide Sodium occurs as white to yellowish white powder.

It is very soluble in dimethylsulfoxide, freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefpiramide Sodium in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ2.3 ppm, at around δ3.9 ppm and at around δ8.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefpiramide Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: -33 – $-40°$ (0.2 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 2.0 g of Cefpiramide Sodium in 20 mL of water is between 5.5 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefpiramide Sodium in 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0: the solution is clear, and colorless or light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cefpiramide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 25 mg of Cefpiramide Sodium, dissolve in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and an amount of Cefpiramide Reference Standard, equivalent to about 75 mg (potency), dissolve them in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL. Pipet 2 mL of this solution, add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances by the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances are not more than 1.0%, not more than 1.5% and not more than 4.0%, respectively.

Amount (%) of 1-methyl-1*H*-tetrazole-5-thiol ($C_2H_4N_4S$)

$$= \frac{W_{Sa}}{W_T} \times \frac{A_{Ta}}{A_{Sa}}$$

Amount (%) of each of other related substances

$$= \frac{W_{Sb}}{W_T} \times \frac{A_{Tc}}{A_{Sb}}$$

W_{Sa}: Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol
W_{Sb}: Amount [mg (potency)] of Cefpiramide Reference Standard
W_T: Amount (mg) of the sample
A_{Sa}: Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the standard solution
A_{Sb}: Peak area of cefpiramide from the standard solution
A_{Ta}: Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the sample solution
A_{Tc}: Area of each peak other than 1-methyl-1*H*-tetrazole-5-thiol and cefpiramide from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.03 mol/L phosphate buffer solution, pH 7.5 and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 11 minutes.

Time span of measurement: About 2 times as long as the retention time of cefpiramide.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazole-5-thiol obtained from 5 μL of this solution is equivalent to 8 to 12% of that from 5 μL of the standard solution.

System performance: Dissolve 25 mg of Cefpiramide Reference Standard and 7 mg of cinnamic acid in the mobile

phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, cinnamic acid and cefpiramide are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazole-5-thiol is not more than 2.0%.

Water Not more than 7.0% (0.35 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefpiramide Sodium and Cefpiramide Reference Standard, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution to dissolve, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefpiramide to that of the internal standard.

Amount [μg (potency)] of cefpiramide ($C_{25}H_{24}N_8O_7S_2$)

$$= W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefpiramide Reference Standard

Internal standard solution—A solution of 4-dimethylaminoantipyrine (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution, pH 6.8, acetonitrile, methanol and tetrahydrofuran (22:1:1:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefpiramide and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefpiramide to that of the internal standard is not more than 2.0%.

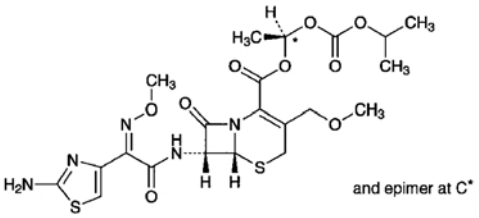
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Add the following:

Cefpodoxime Proxetil

セフポドキシムプロキセチル



$C_{21}H_{27}N_5O_9S_2$: 557.60

(1*RS*)-1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [*87239-81-4*]

Cefpodoxime Proxetil contains not less than 706 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpodoxime Proxetil is expressed as mass (potency) of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Description Cefpodoxime Proxetil occurs as a white to light brownish white powder.

It is very soluble in acetonitrile, in methanol and in chloroform, freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefpodoxime Proxetil in acetonitrile (3 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefpodoxime Proxetil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefpodoxime Proxetil as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefpodoxime Proxetil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefpodoxime Proxetil in deuterochloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits double signals, A and B, at around δ1.3 ppm and at around δ1.6 ppm, and single signals, C and D, at around δ3.3 ppm and at around δ4.0 ppm. The ratio of the integrated intensity of these signals, A:B:C:D, is about 2:1:1:1.

Optical rotation $[\alpha]_D^{20}$: +24.0 – +31.4° (0.1 g calculated on the anhydrous basis, acetonitrile, 20 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Cefpodoxime Proxetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. If necessary, perform the test in the same manner with 20 μL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to compensate for the base line. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the peak, having the relative retention time of about 0.8 with respect to the isomer B of cefpodoxime proxetil, is not more than 2.0%, the peak other than cefpodoxime proxetil is not more than 1.0%, and the sum of the peaks other than cefpodoxime proxetil is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: A mixture of water, methanol and a solution of formic acid (1 in 50) (11:8:1).

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 65	95	5
65 – 145	95 → 15	5 → 85
145 – 155	15	85

Flow rate: Adjust the flow rate so that the retention time of the isomer B of cefpodoxime proxetil is about 60 minutes.

Time span of measurement: About 2.5 times as long as the retention time of the isomer B of cefpodoxime proxetil after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 200 mL, and use this solution as the solution for required detectability test. Pipet 2 mL of the solution for required detectability test, and add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Confirm that the peak areas of the isomer A and the isomer B of cefpodoxime proxetil obtained from 20 μL of this solution are equivalent to 1.4 to 2.6% of them from 20 μL of the solution for required detectability test, respectively.

System performance: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2). When the procedure is run with 20 μL of this solution under the above operating conditions, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2). When the test is repeated 5 times with 20 μL of this solution under the above operating conditions, the relative standard deviations of the peak areas of the isomer A and the isomer B are not more than 2.0%, respectively.

Water Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.20% (1 g).

Isomer ratio Perform the test with 5 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_a and A_b, of the two isomers of cefpodoxime proxetil, having the smaller and larger retention times, respectively, by the automatic integration method: $A_b/(A_a + A_b)$ is between 0.50 and 0.60.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately an amount of Cefpodoxime Proxetil and Cefpodoxime Proxetil Reference Standard, equivalent to about 60 mg (potency), dissolve in 80 mL of acetonitrile, add exactly 4 mL of the internal standard solution, add acetonitrile to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_{T1}, Q_{S1}, Q_{T2} and Q_{S2}, of the areas of the two peaks of the isomers of cefpodoxime proxetil to the peak area of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefpodoxime } (C_{15}H_{17}N_5O_6S_2) = W_S \times \frac{Q_{T1} + Q_{T2}}{Q_{S1} + Q_{S2}} \times 1000$$

W_S: Amount [mg (potency)] of Cefpodoxime Proxetil Reference Standard

Internal standard solution—Dissolve 0.3 g of ethyl parahydroxybenzoate in a solution of citric acid in acetonitrile (1 in 2000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (11:9).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 11 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard, the isomer A and the isomer B are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of the isomer B of cefpodoxime proxetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefroxadine

セフロキサジン

Change to read except the structural formula and chemical name:

Cefroxadine contains not less than 921 μg (potency) per mg, calculated on the dehydrated basis. The potency of Cefroxadine is expressed as mass (potency) of cefroxadine ($C_{16}H_{19}N_3O_5S$: 365.41).

Description Cefroxadine occurs as pale yellowish white to light yellow, crystalline particles or powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, and very slightly soluble in acetonitrile and in ethanol (95).

It dissolves in 0.001 mol/L hydrochloric acid TS and in dilute acetic acid.

Identification **(1)** Determine the absorption spectrum of a solution of Cefroxadine in 0.001 mol/L hydrochloric acid TS (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefroxadine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefroxadine in deuterated formic acid for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three sharp single signals, A, B and C, at around δ2.8 ppm, at around δ4.1 ppm and at around δ6.3 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 4:3:1.

Optical rotation $[\alpha]_D^{20}$: +95 – +108° (0.1 g calculated on the dehydrated basis, diluted acetic acid (100) (3 in 25), 100 mL, 100 mm).

Purity **(1)** Heavy metals—Weigh 1.0 g of Cefroxadine in a porcelain crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), mix, burn the ethanol, and carbonize by gently heating. After cooling, add 2 mL of nitric acid, heat carefully, and incinerate by ignition at 500 – 600°C. If a carbonized substance is still remained, moisten it with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Moisten the residue with 3 drops of hydrochloric acid, and add 10 mL of hot water to dissolve the residue by heating on a water bath. After cooling, adjust the pH between 3 and 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, transfer to a Nessler tube, wash the crucible with 10 mL of water, and add the washing and water to the tube to make 50 mL. Perform the test with this solution. Prepare the control solution as follows: Put 2.0 mL of Standard Lead Solution and 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) in a porcelain crucible, and proceed as directed for the preparation of the test solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Cefroxadine according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 10 mg of Cefroxadine in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: the areas of the peaks appeared at the relative retention times of 0.07, 0.6 and 0.8 against the peak of cefroxadine from the sample solution are not more than 2 times, 4 times and 1 time of the peak area of cefroxadine from the standard solution, respectively, and any peak area other than cefroxadine and other than the peaks mentioned above from the sample solution is not more than 1/2 of the peak area of cefroxadine from the standard solution, and the total area of the peaks other than cefroxadine from the sample solution is not more than 6 times of the peak area of cefroxadine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.4 g of sodium perchlorate in 1000 mL of a mixture of water and acetonitrile (489:11).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of cefroxadine.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefroxadine obtained from 40 μL of this solution is equivalent to 7 to 13% of that obtained from 40 μL of the standard solution.

System performance: Dissolve 3 mg of Cefroxadine and 15 mg of orcin in 100 mL of the mobile phase. When the procedure is run with 40 μL of this solution under the above operating conditions, orcin and cefroxadine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefroxadine is not more than 2.0%.

Water Not less than 8.5% and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefroxadine and Cefroxadine Reference Standard, equivalent to about 50 mg (potency), dissolve each in a suitable amount of a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution and a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefroxadine to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{16}H_{19}N_3O_5S = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefroxadine Reference Standard

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 50) and acetonitrile (97:3).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefroxadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefroxadine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefteram Pivoxil

セフテラムピボキシル

Change to read except the structural formula and chemical name:

Cefteram Pivoxil contains not less than 743 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefteram Pivoxil is expressed as mass (potency) of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Description Cefteram Pivoxil occurs as a white to pale yellowish white powder.

It is very soluble in acetonitrile, freely soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B and C, at around δ1.2 ppm, at around δ2.5 ppm and at around δ4.0 ppm, respectively. The ratio of the integrated intensity of these signals, A:B:C, is about 3:1:1.

Optical rotation $[\alpha]_D^{20}$: +35 – +43° (0.4 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Cefter-

am Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Cefteram Pivoxil according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Cefteram Pivoxilin in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil from the sample solution is not more than 1.25 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil from the sample solution is not more than 0.25 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil from the sample solution is not more than 2.75 times the peak area of cefteram pivoxil from the standard solution. For the above calculation, use the area of the peak, having the relative retention time of about 0.1, after multiplying by its sensitivity coefficient, 0.74.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefteram pivoxil is not more than 3.0%.

Water Not more than 3.0% (0.3 g, coulometric titration).

Assay Weigh accurately an amount of Cefteram Pivoxil and Cefteram Pivoxil Mesitylenesulfonate Reference Standard, equivalent to about 40 mg (potency), dissolve each in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of cefteram pivoxil to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefteram } (C_{16}H_{17}N_9O_5S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylenesulfonate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0 add 375 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefteram pivoxil is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefteram pivoxil are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefteram pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Ceftibuten

セフチブテン

Change the Identification (1) to read:

Identification (1) Determine the absorption spectrum of a solution of Ceftibuten in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 261 nm and 265 nm.

Change the Absorbance to read:

Absorbance $E^{1\%}_{1\,cm}$ (263 nm): 320 – 345 (20 mg calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution

for antibiotics, pH 8.0, 1000 mL).

Change the Optical rotation to read:

Optical rotation $[\alpha]_D^{20}$: +135 – +155° (0.3 g calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, 50 mL, 100 mm).

Change the Assay to read:

Assay Weigh accurately an amount of Ceftibuten and Ceftibuten Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, add exactly 4 mL each of the internal standard solution, shake, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftibuten to that of the internal standard. Keep the sample solution and the standard solution at 5°C or below and use within 2 hours.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{14}N_4O_6S_2 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Ceftibuten Hydrochloride Reference Standard

Internal standard solution—A solution of methyl *p*-hydroxybenzoate in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.005 mol/L *n*-decyl trimethylammonium bromide TS and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ceftibuten is about 10 minutes.

System suitability—

System performance: Dissolve 5 mg of Ceftibuten in 1 mol/L Hydrochloric acid TS to make 50 mL, and allow to stand for 4 hours at room temperature. To 10 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer and ceftibuten are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftibuten to that of the internal standard is not more than 1.0%.

Ceftriaxone Sodium

セフトリアキソンナトリウム

Change the Identification (1) to read:

Identification **(1)** Determine the absorption spectrum of a solution of Ceftriaxone Sodium (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftriaxone Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Add the following next to Purity (3):

Purity

(4) Related substances 1—Dissolve 20 mg of Ceftriaxone Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the impurity 1 having the relative retention time of about 0.5 and the impurity 2 having the relative retention time of about 1.3 to ceftriaxone from the sample solution are not more than the peak area of ceftriaxone from the standard solution. In this case, these peak areas for the impurity 1 and the impurity 2 are used after multiplying by 0.9 and 1.2, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, add 55 mL of the solution A, 5 mL of the solution B and 490 mL of water.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium in a mixture of water and acetonitrile for liquid chromatography (11:9) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order, with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

(5) Related substances 2—Dissolve 10 mg of Ceftriaxone Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the each peak area of the impurities which appear after the peak of ceftriaxone from the sample solution is not more than the peak area of ceftriaxone from the standard solution, and the total peak area of these impurities is not more than 2.5 times of the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 55 mL of the solution A, 5 mL of the solution B, 490 mL of water and 700 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 3 minutes.

Time span of measurement: About 10 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as the solution for system suitability test. Measure exactly 1 mL of the solution for system suitability test, and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium in a mixture of acetonitrile for liquid chromatography and water (23:11) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

Change the Assay to read:

Assay Weigh accurately an amount of Ceftriaxone Sodium and Ceftriaxone Sodium Reference Standard, equivalent to about 0.1 g (potency), dissolve each in a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 50 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftriaxone to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ceftriaxone } (C_{18}H_{18}N_8O_7S_3) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Ceftriaxone Sodium Reference Standard

Internal standard solution—A solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0%.

Cefuroxime Axetil

セフロキシムアキセチル

Change to read except the structural formula and chemical name:

Cefuroxime Axetil contains not less than 760 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of acetone. The potency of Cefuroxime Axetil is expressed as mass (potency) of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39).

Description Cefuroxime Axetil occurs as white to yellowish white, non-crystalline powder.

It is freely soluble in dimethylsulfoxide, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefuroxime Axetil in methanol (3 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefuroxime Axetil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefuroxime Axetil as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Cefuroxime Axetil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefuroxime Axetil in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal or a pair of double signals A at around δ1.5 ppm, a pair of single signals B at around δ2.1 ppm, and a single signal C at around δ3.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 1:1:1.

Optical rotation $[\alpha]_D^{20}$: +41 – +47° (0.5 g, methanol, 50 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 2.0 g of Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Put 1.0 g of Cefuroxime Axetil in a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, then heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substance—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 40 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the sample solution is not more than 1.5 times the sum area of two peaks of cefuroxime axetil obtained from the standard solution, and the sum area of the peaks other than cefuroxime axetil from the sample solution is not more than 4 times the sum area of two peaks of cefuroxime axetil from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the

retention time of the peak having the larger retention time of the two peaks of cefuroxime axetil after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 10 mL. Confirm that the sum area of the two peaks of cefuroxime axetil obtained from 2 μL of this solution is equivalent to 7 to 13% of that obtained from 2 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the sum area of the two peaks of cefuroxime axetil is not more than 2.0%.

(4) Acetone—Weigh accurately about 1.0 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratios, Q_T and Q_S, of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3%.

$$\text{Amount (\%) of acetone} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times 0.2$$

W_S: Amount (g) of acetone

W_T: Amount (g) of the sample

Internal standard solution—A solution of 1-propanol in dimethylsulfoxide (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1:1) in the ratio of 20% (125 – 150 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Temperature of injection port: A constant temperature of about 115°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 4 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of acetone to that of the internal standard is not more than 5.0%.

Water Not more than 2.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.20% (0.5 g).

Isomer ratio Perform the test with 10 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions, and determine the area, A_a, of the peak having the smaller retention time and the area, A_b, of the peak having the bigger retention time of the two peaks of cefuroxime axetil:

$A_b/(A_a + A_b)$ is between 0.48 and 0.55.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately an amount of Cefuroxime Axetil and Cefuroxime Axetil Reference Standard, equivalent to about 50 mg (potency), and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, 5 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard.

$$\text{Amount [μg (potency)] of cefuroxime } (C_{16}H_{16}N_4O_8S) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Cefuroxime Axetil Reference Standard

Internal standard solution—A solution of acetanilide in methanol (27 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium dihydrogen phosphate (23 in 1000) and methanol (5:3).

Flow rate: Adjust the flow rate so that the retention time of the peak having the smaller retention time of the two peaks of cefuroxime axetil is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cetraxate Hydrochloride

塩酸セトラキサート

Change the Purity (3) and (4) to read:

Purity

(3) *cis* Isomer—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of water, and use this solution as the sample solution. To exactly 5 mL of the sample solution add water to make exactly 100 mL. To exactly 2 mL of this solution add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak which has a retention time 1.3 to 1.6 times that of cetraxate from the sample solution is not larger than the peak area of cetraxate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:10:4) to 6.0 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of cetraxate is about 10 minutes.

System suitability—

System performance: Dissolve 0.02 g of Cetraxate Hydrochloride and 0.01 g of phenol in 100 mL of water. To 2 mL of this solution add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetraxate and phenol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetraxate is not more than 2.0%.

(4) 3-(*p*-Hydroxyphenyl)propionic acid—To 0.10 g of Cetraxate Hydrochloride add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 3-(*p*-hydroxyphenyl)propionic acid in methanol to make exactly 100 mL. To exactly 2 mL of this solution add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of caffeine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:5:2) to 5.5 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of 3-(*p*-hydroxyphenyl)propionic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 3-(*p*-hydroxyphenyl)propionic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard is not more than 1.0%.

Chloramphenicol

クロラムフェニコール

Change to read except the structural formula and chemical name:

Chloramphenicol contains not less than 980 μg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

Description Chloramphenicol occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Chloramphenicol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +18.5 – +21.5° (1.25 g, ethanol (99.5), 25 mL, 100 mm).

Melting point 150 – 155°C

Purity **(1)** Heavy metals—Proceed with 1.0 g of Chloramphenicol according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Arsenic—Prepare the test solution with 2.0 g of Chloramphenicol according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Chloramphenicol in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of chloroform, methanol and acetic acid (100) (79:14:7) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot on the original obtained from the sample solution are not more intense than the spot from the standard solution (1), and the total amount of these spots is not more than 2.0%.

Loss on drying Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately an amount of Chloramphenicol and Chloramphenicol Reference Standard, equivalent to about 0.1 g (potency), dissolve each in 20 mL of methanol, and add water to make exactly 100 mL. Pipet 20 mL each of these solutions, and add water to make exactly 100 mL. Pipet 10 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount [}\mu\text{g (potency)] of } C_{11}H_{12}Cl_2N_2O_5 = W_S \times \frac{A_T}{A_S} \times 1000$$

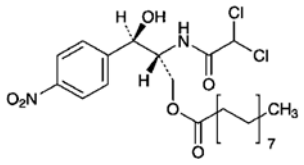
W_S: Amount [mg (potency)] of Chloramphenicol Reference Standard

Containers and storage Containers—Tight containers.

Add the following:

Chloramphenicol Palmitate

パルミチン酸クロラムフェニコール



$C_{27}H_{42}Cl_2N_2O_6$: 561.54

(2*R*,3*R*)-2-(Dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl palmitate [*530-43-8*]

Chloramphenicol Palmitate contains not less than 558 μg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol Palmitate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Palmitate occurs as a white to grayish white, crystalline powder.

It is freely soluble in acetone, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Chloramphenicol Palmitate in ethanol (99.5) (1 in 33,000) as directed under the Ultraviolet-visible Spec-

trophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Palmitate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Chloramphenicol Palmitate and Chloramphenicol Palmitate Reference Standard in 1 mL of acetone, and use these solutions as the sample solution and the standard soution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same *R*f value as the spot from the standard solution.

Optical rotation $[\alpha]_D^{25}$: +21 – +25° (1 g calculated on the dried basis, ethanol (99.5), 20 mL, 100 mm).

Melting point 91 – 96°C

Purity **(1)** Heavy metals—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Chloramphenicol Palmitate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. The test should be performed within 30 minutes after the sample solution and the standard solution are prepared. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of chloramphenicol palmitate from the sample solution is not more than 3.5 times the peak area of chloramphenicol palmitate from the standard solution. For this calculation, use the peak areas for chloramphenicol, having the relative retention time of about 0.5 with respect to chloramphenicol palmitate, and for chloramphenicol dipalmitate, having the relative retention time of about 5.0 with respect to chloramphenicol palmitate, after multiplying by their response factors, 0.5 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of chloramphenicol palmitate.

System suitability—

Test for required detectability: Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitabillity test, and add methanol to make exactly 50 mL. Confirm that the peak area of chloramphenicol palmitate obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 5000.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Loss on drying Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Chloramphenicol Palmitate and Chloramphenicol Palmitate Reference Standard, equivalent to about 37 mg (potency), dissolve each in 40 mL of methanol and exactly 1 mL of acetic acid (100), and add methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S.

Amount [μg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)

$$= W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Chloramphenicol Palmitate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water and acetic acid (100) (172:27:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 2400.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

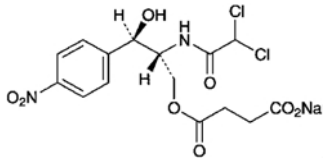
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Chloramphenicol Sodium Succinate

コハク酸クロラムフェニコールナトリウム



$C_{15}H_{15}Cl_2N_2NaO_8$: 445.18

Monosodium (2*R*,3*R*)-2-(dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl succinate

[*982-57-0*]

Chloramphenicol Sodium Succinate contains not less than 711 μg (potency) per mg, calculated on the anhydrous basis. The potency of Chloramphenicol Sodium Succinate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Sodium Succinate occurs as white to yellowish white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Chloramphenicol Sodium Succinate (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol Sodium Succinate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Chloramphenicol Sodium Succinate responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{25}$: +5 – +8° (1.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.4 g of Chloramphenicol Sodium Succinate in 5 mL of water is between 6.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear and colorless to yellowish.

(2) Heavy metals—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Chloramphenicol Sodium Succinate according to Method 1, and perform the test (not more than 2 ppm).

Water Not more than 2.0% (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Chloramphenicol Sodium Succinate, equivalent to about 20 mg (potency), dissolve in water to make exactly 1000 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Chloramphenicol Succinate Reference Standard, equivalent to about 20 mg (potency), add about 50 mL of water to make a suspension, and add gradually about 7 mL of 0.01 mol/L sodium hydroxide TS while stirring to adjust the pH to 7.0. To this solution add water to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 276 nm of the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry.

Amount [μg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)

$$= W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Chloramphenicol Succinate Reference Standard

Containers and storage Containers—Hermetic containers.

Chlordiazepoxide Powder

クロルジアゼポキシド散

Change the Assay to read:

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Powder, equivalent to about 0.1 g of Chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, wet with exactly 10 mL of water, add exactly 90 mL of methanol, stopper, shake vigorously for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add

methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Chlordiazepoxide Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, and dissolve in exactly 10 mL of water and 90 mL of methanol. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlordiazepoxide to that of the internal standard.

$$\text{Amount (mg) of chlordiazepoxide } (C_{16}H_{14}ClN_3O) = W_S \times \frac{Q_T}{Q_S} \times 10$$

W_S: Amount (mg) of Chlordiazepoxide Reference Standard

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Change the Assay to read:

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Tablets, equivalent to about 0.1 g of Chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Chlordiazepoxide Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and a suitable amount of methanol, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlordiazepoxide to that of the internal standard.

$$\text{Amount (mg) of chlordiazepoxide } (C_{16}H_{14}ClN_3O) = W_S \times \frac{Q_T}{Q_S} \times 10$$

W_S: Amount (mg) of Chlordiazepoxide Reference Standard

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Chlormadinone Acetate

酢酸クロルマジノン

Change the Purity (3) to read:

Purity

(3) Other steroids—Dissolve 20 mg of Chlormadinone Acetate in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add

acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the peak of chlormadinone acetate from the sample solution is not larger than the peak area of chlormadinone acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 236 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of acetonitrile and water (13:7).

Flow rate: Adjust the flow rate so that the retention time of chlormadinone acetate is about 10 minutes.

Time span of measurement: About 1.5 times as long as the retention time of chlormadinone acetate after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add acetonitorile to make exactly 50 mL. Confirm that the peak area of chlormadinone acetate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of chlormadinone acetate obtained from 10 μL of the standard solution.

System performance: Dissolve 8 mg of Chlormadinone Acetate and 2 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and chlormadinone acetate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlormadinone acetate is not more than 1.0%.

Chlorphenesin Carbamate

カルバミン酸クロルフェネシン

Change the Purity (3) to read:

Purity

(3) Related substances—(i) Chlorphenesin-2-carbamate: Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of hexane for liquid chromatography and 2-propanol (7:3), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak area, A_a, of chlorphenesin carbamate and the peak area, A_b, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, $A_b/(A_a + A_b)$, is not larger than 0.007.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 5 mL of the solution for system suitability test add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 10 mL. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is equivalent to 40 to 60% of that of chlorphenesin carbamate obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in 50 mL of methanol. To 25 mL of this solution add 25 mL of dilute sodium hydroxide TS, and warm at 60°C for 20 minutes. To 20 mL of this solution add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethyl acetate, and allow to stand to separate the ethyl acetate layer. When the procedure is run with 10 μL of this layer under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2-carbamate are eluted in this order, with the ratios of the retention time of chlorphenesin and chlorphenesin-2-carbamate with respect to chlorphenesin carbamate are about 0.7 and about 1.2, respectively, and with the resolution between the peaks of chlorphenesin and chlorphenesin carbamate being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chlorphenesin carbamate is not more than 2.0%.

(ii) Other related substances: Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 50 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate.

Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Ciclacillin

シクラシリン

Change to read except the structural formula and chemical name:

Ciclacillin contains not less than 920 μg (potency) per mg, calculated on the dehydrated basis. The potency of Ciclacillin is expressed as mass (potency) of ciclacillin ($C_{15}H_{23}N_3O_4S$).

Description Ciclacillin occurs as white to light yellowish white crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Ciclacillin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclacillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +300 – +315° (2 g, water, 100 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Ciclacillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Ciclacillin according to Method 3, and perform the test (not more than 2 ppm).

Water Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ciclacillin and Ciclacillin Reference Standard, equivalent to about 50 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of ciclacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{23}N_3O_4S = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Ciclacillin Reference Standard

Internal standard solution—A solution of orcin in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Citric Acid

クエン酸

Change to read except the structural formula and chemical name:

Citric Acid contains not less than 99.5% and not more than 100.5% of anhydrous citric acid ($C_6H_8O_7$: 192.12), calculated on the anhydrous basis.

Description Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is efflorescent in dry air.

Identification Determine the infrared absorption spectrum of Citric Acid, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Citric Acid in water to make 10 mL: the solution is clear and has no more color than the following control solutions

(1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution add water to make 1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 7.2 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.15 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 1.0 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalate—Dissolve 0.80 g of Citric Acid in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium hydrochloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time.

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

(4) Heavy metals—Proceed with 2.0 g of Citric Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Readily carbonizable substances—Perform the test with 0.5 g of Citric Acid, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water Not less than 7.5% and not more than 9.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.55 g of Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

Containers and storage Containers—Tight containers.

Anhydrous Citric Acid

無水クエン酸

Change to read except the structural formula and chemical name:

Anhydrous Citric Acid contains not less than 99.5% and not more than 100.5% of $C_6H_8O_7$, calculated on the anhydrous basis.

Description Anhydrous Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Anhydrous Citric Acid, previously dried at 105°C for 24 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 10 mL: the solution is clear and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution add water to make 1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 7.2 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.15 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 1.0 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acet-

ic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalate—Dissolve 0.80 g of Anhydrous Citric Acid in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium hydrochloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time.

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

(4) Heavy metals—Proceed with 2.0 g of Anhydrous Citric Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Readily carbonizable substances—Perform the test with 0.5 g of Anhydrous Citric Acid, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

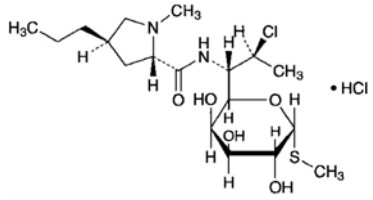
Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

Containers and storage Containers—Tight containers.

Add the following:

Clindamycin Hydrochloride

塩酸クリンダマイシン



$C_{18}H_{33}ClN_2O_5S.HCl$: 461.44
Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside monohydrochloride [*21462-39-5*]

Clindamycin Hydrochloride contains not less than 759 μg (potency) per mg. The potency of Clindamycin Hydrochloride is expressed as mass (potency) of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Description Clindamycin Hydrochloride occurs as white to grayish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (95).

Identification Dissolve 0.1 g of Clindamycin Hydrochloride in 5 mL of water, add 2 mL of sodium hydroxide TS, and mix: a white turbidity is produced. To this solution add 0.3 mL of sodium pentacyanonitrosylferrate (III) TS, mix, allow to stand at 60 to 65°C for 10 minutes, and add 2 mL of dilute hydrochloric acid: a blue-green color develops.

Optical rotation $[\alpha]_D^{25}$: +135 – +150° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Water Not more than 6.0% (0.3 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Micrococcus luteus* ATCC 9341

(2) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Clindamycin Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 250 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 15°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 2 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of

Clindamycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 250 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 2 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Clindamycin Phosphate

リン酸クリンダマイシン

Change to read except the structural formula and chemical name:

Clindamycin Phosphate contains not less than 758 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Phosphate is expressed as mass (potency) of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Description Clindamycin Phosphate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Clindamycin Phosphate, previously dried at 100°C for 2 hours, as directed in the paste method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Phosphate Reference Standard previously dried at 100°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation $[\alpha]_D^{20}$: +115 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH Dissolve 0.10 g of Clindamycin Phosphate in 10 mL of water. The pH of the solution is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Clindamycin Phosphate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 2.0 g of Clindamycin Phosphate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Clindamycin Phosphate according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Clindamycin Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of clindamycin, having the relative retention time of about 1.8 with respect to clindamycin phosphate, obtained from the sample solution is not more than 1/2 of the peak area of clindamycin phosphate from the standard solution, and the total area of the peaks other than clindamycin phosphate from the sample solution is not more than 4 times the peak area of clindamycin phosphate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of clindamycin phosphate after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin phosphate obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Clindamycin Phosphate and Clindamycin Phosphate Reference Standard, equivalent to about 20 mg (potency), add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Clindamycin Phosphate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10.54 g of potassium dihydrogen

phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin phosphate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5%.

Containers and storage Containers—Tight containers.

Clomifene Citrate

クエン酸クロミフェン

Change the Isomer ratio to read:

Isomer ratio To 0.10 g of Clomifene Citrate add 10 mL of water and 1 mL of sodium hydroxide TS, and extract with three 15-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 20 mL of water, add 10 g of anhydrous sodium sulfate to the combined diethyl ether extracts, shake for 1 minute, filter, and evaporate the diethyl ether of the filtrate. Dissolve the residue in 10 mL of chloroform, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under the Gas Chromatography according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having retention times of about 20 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a + A_b)$ is between 0.3 and 0.5.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, having methylsilicone polymer coated at the ratio of 1% on siliceous earth for gas chromatography (125 to 150 μm in particle diameter).

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the first peak of clomifene citrate is about 20 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the sample solution under the above operating conditions, the resolution between the two peaks is not less than 1.3.

System repeatability: When the test is repeated 5 times with 2 μL of the sample solution under the above operating conditions, the relative standard deviation of $A_b/(A_a + A_b)$ is not more than 5.0%.

Cloxacillin Sodium

クロキサシリンナトリウム

Change the Description to read:

Description Cloxacillin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, in *N*,*N*-dimethylformamide and in methanol, and sparingly soluble in ethanol (95).

Change the Identification (1) to read:

Identification (1) Determine the absorption spectrum of a solution of Cloxacillin Sodium in methanol (1 in 2500) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cloxacillin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelength.

Add the following next to Identification:

Optical rotation $[\alpha]_D^{20}$: +163 – +171° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Change the pH to read:

pH Dissolve 1.0 g of Cloxacillin Sodium in 10 mL of water: the pH of the solution is between 6.0 and 7.5.

Change the Purity to read:

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cloxacillin Sodium in 10 mL of water is clear and colorless to light yellow.

(2) Heavy metals—Proceed with 1.0 g of Cloxacillin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Cloxacillin Sodium according to Method 5, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Cloxacillin Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cloxacillin obtained from the sample solution is not more than the peak area of cloxacillin obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter

and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.953 g of diammonium hydrogen phosphate in 700 mL of water, and add 250 mL of acetonitrile. Adjust the pH to 4.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cloxacillin is about 24 minutes.

Time span of measurement: About 3 times as long as the retention time of cloxacillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cloxacillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from the standard solution.

System performance: Weigh accurately about 50 mg of Cloxacillin Sodium Reference Standard, dissolve in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), then add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of cloxacillin to that of guaifenesin is not more than 1.0%.

Change the Water to read:

Water 3.0 – 4.5% (0.2 g, volumetric titration, direct titration).

1% Codeine Phosphate Powder

リン酸コデイン散 1%

Change the Assay to read:

Assay Weigh accurately about 5 g of 1% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay, separately determined the water in the same manner as Codeine Phosphate, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard.

$$\text{Amount (mg) of codeine phosphate } (C_{18}H_{21}NO_3.H_3PO_4.\tfrac{1}{2}H_2O) = W_S \times \frac{Q_T}{Q_S} \times 1.0227$$

W_S: Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

10% Codeine Phosphate Powder

リン酸コデイン散 10%

Change the Assay to read:

Assay Weigh accurately about 2.5 g of 10% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay, separately determined the water in the same manner as Codeine Phosphate, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the

following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard:

$$\text{Amount (mg) of codeine phosphate } (C_{18}H_{21}NO_3.H_3PO_4.\tfrac{1}{2}H_2O) = W_S \times \frac{Q_T}{Q_S} \times 5 \times 1.0227$$

W_S: Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Codeine Phosphate Tablets

リン酸コデイン錠

Change the Assay to read:

Assay Weigh accurately and powder not less than 20 Codeine Phosphate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of codeine phosphate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$), add 30 mL of water, shake, add 20 mL of diluted dilute sulfuric acid (1 in 20), treat the mixture with ultrasonic waves for 10 minutes, and add water to make exactly 100 mL. Filter the solution, then pipet 5 mL of the filtrate, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay, separately determined its water content in the same manner as Codeine Phosphate, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard.

$$\text{Amount (mg) of codeine phosphate } (C_{18}H_{21}NO_3.H_3PO_4.\tfrac{1}{2}H_2O) = W_S \times \frac{Q_T}{Q_S} \times 2 \times 1.0227$$

W_S: Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

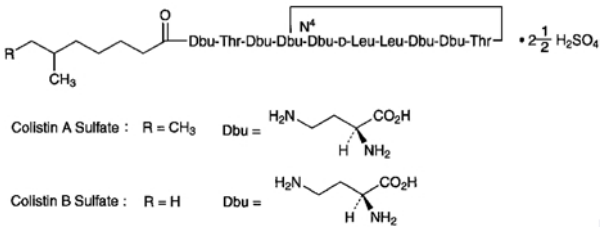
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Add the following:

Colistin Sulfate

硫酸コリスチン



Colistin A Sulfate $C_{53}H_{100}N_{16}O_{13}.2\frac{1}{2}H_2SO_4$: 1414.66

Colistin B Sulfate $C_{52}H_{98}N_{16}O_{13}.2\frac{1}{2}H_2SO_4$: 1400.63

[*1264-72-8*]

Colistin Sulfate, when dried, contains not less than 16,000 units per mg. The potency of Colistin Sulfate is expressed as unit calculated from the amount of colistin A ($C_{53}H_{100}N_{16}O_{13}$: 1169.46). One unit of Colistin Sulfate is equivalent to 0.04 μg of colistin A ($C_{53}H_{100}N_{16}O_{13}$).

Description Colistin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 20 mg of Colistin Sulfate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, then add 5 drops of copper (II) sulfate TS while shaking: a purple color develops.

(2) Dissolve 50 mg of Colistin Sulfate in 10 mL of diluted hydrochloric acid (1 in 2). Transfer 1 mL of this solution in a tube for hydrolysis, seal, and heat at 135°C for 5 hours. After cooling, open the tube, and evaporate the content to dryness until the odor of hydrochloric acid is no more perceptible. Dissolve the residue in 0.5 mL of water, and use this solution as the sample solution. Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine in 10 mL of water, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (60:15:10:6:5) to a distance of about 10 cm, and dry the plate at 105°C for 10 minutes. Spray evenly ninhydrin TS on the plate, and heat at 110°C for 5 minutes: three principal spots are obtained from the sample solution, the *R*f values of two spots of them are the same with those of the corresponding spots obtained from the standard solution (1) and the standard solution (2), and the *R*f value of the rest principal spot is about 0.1. No spot is observed at the position corresponding to the spots obtained from the standard solution (3) and the standard solution (4).

(3) A solution of Colistin Sulfate (1 in 20) responds to the Qualitative Test (1) for sulfate.

Optical rotation $[\alpha]_D^{20}$: -63 – $-73°$ (1.25 g, after drying, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Colistin Sulfate in 10 mL of water is between 4.0 and 6.0.

Purity **(1)** Sulfuric acid—Weigh accurately about 0.25 g of previously dried Colistin Sulfate, dissolve in a suitable amount of water, adjust the pH to 11 with ammonia solution (28), and add water to make 100 mL. To this solution add exactly 10 mL of 0.1 mol/L barium chloride VS and 50 mL of ethanol (99.5), and titrate with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue-purple color of the solution disappears (indicator: 0.5 mg of phthalein purple): the amount of sulfuric acid (SO_4) is 16.0 to 18.0%.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of SO_4

(2) Related substances – Dissolve 50 mg of Colistin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of pyridine, 1-butanol, water and acetic acid (100) (6:5:4:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 100°C for about 20 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Escherichia coli* NIHJ

(2) Culture medium—Dissolve 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 15.0 g of agar in 1000 mL of water, adjust the pH with sodium hydroxide TS so that the solution will be 6.5 to 6.6 after sterilization, and use as the agar media for seed layer and for base layer.

(3) Standard solutions—Weigh accurately an amount of Colistin Sulfate Reference Standard, previously dried, equivalent to about 1,000,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL, and use this solution as the standard stock solution. Keep the standard

stock solution at not exceeding 10°C, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

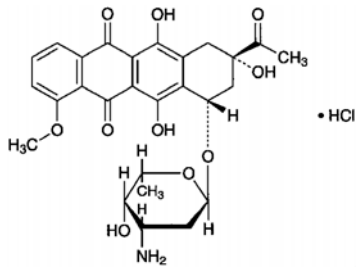
(4) Sample solutions—Weigh accurately an amount of Colistin Sulfate, previously dried, equivalent to about 1,000,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Add the following:

Daunorubicin Hydrochloride

塩酸ダウノルビシン



$C_{27}H_{29}NO_{10}$.HCl: 563.98

(2*S*,4*S*)-2-Acetyl-4-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-1,2,3,4-tetrahydro-2,5,12-trihydroxy-7-methoxynaphthacene-6,11-dione monohydrochloride [*23541-50-6*]

Daunorubicin Hydrochloride contains not less than 940 μg (potency) per mg, calculated on the dried basis. The potency of Daunorubicin Hydrochloride is expressed as mass (potency) of daunorubicin hydrochloride ($C_{27}H_{29}NO_{10}$.HCl).

Description Daunorubicin Hydrochloride occurs as a red powder.

It is soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Daunorubicin Hydrochloride in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Daunorubicin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Test (2) for chloride.

Optical rotation $[\alpha]_D^{20}$: +250 – +275° (15 mg calculated on the dried basis, methanol, 10 mL, 100 mm).

pH Dissolve 0.15 g of Daunorubicin Hydrochloride in 30 mL of water: the pH of the solution is between 4.5 and 6.0.

Purity (1) Clarity and color of solution – Dissolve 20 mg of Daunorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Heavy metals—Proceed with 1.0 g of Daunorubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Daunorubicin Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water and acetic acid (100) (15:5:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the naked eye: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 7.5% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of daunorubicin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{27}H_{29}NO_{10}\text{.HCl} = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Daunorubicin Hydrochloride Reference Standard

Internal standard solution—A solution of 2-naphthalenesulfonic acid in the mobile phase (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water and acetonitrile (31:19) to 2.2 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of daunorubicin is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and daunorubicin are eluted in this order with the resolution between these peaks being not less than 2.0.

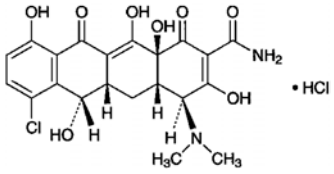
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of daunorubicin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Add the following:

Demethylchlortetracycline Hydrochloride

塩酸デメチルクロルテトラサイクリン



$C_{21}H_{21}ClN_2O_8$.HCl: 501.31

(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-1,11-dioxonaphthacene-2-carboxamide monohydrochloride [*64-73-3*]

Demethylchlortetracycline Hydrochloride contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Demethylchlortetracycline Hydrochloride is expressed as mass (potency) of demethylchlortetracycline hydrochloride ($C_{21}H_{21}ClN_2O_8$.HCl).

Description Demethylchlortetracycline Hydrochloride occurs as a yellow crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Dissolve 40 mg of Demethylchlortetracycline Hydrochloride in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Demethylchlortetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Demethylchlortetracycline Hydrochloride as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Demethylchlortetracycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Demethylchlortetracycline Hydrochloride (1 in 100) responds to the Qualitative Test (2) for chloride.

Optical rotation $[\alpha]_D^{20}$: −248 – −263° (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 1.0 g of Demethylchlortetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 2.0 and 3.0.

Purity (1) Heavy metals—Proceed with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Demethylchlortetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: the peak area other than demethylchlortetracycline obtained from the sample solution is not more than 6/5 times that of demethylchlortetracycline from the standard solution, and the sum of the areas of the peaks other than demethylchlortetracycline from the sample solution is not more than 2 times the peak area of demethylchlortetracycline from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of demethylchlortetracycline after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 10 mL of the standard solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the solution

for system suitability test. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of demethylchlortetracycline obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Loss on drying Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Demethylchlortetracycline Hydrochloride and Demethylchlortetracycline Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve each in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of demethylchlortetracycline.

$$\text{Amount [}\mu\text{g (potency)] of } C_{21}H_{21}ClN_2O_8.HCl = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Demethylchlortetracycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate, 1.5 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, and adjust the pH to 8.5 with sodium hydroxide TS. To this solution add 75.0 g of *t*-butanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of demethylchlortetracycline is about 8 minutes.

System suitability—

System performance: Heat 10 mL of the standard solution on a water bath for 60 minutes. When the procedure is run with 20 μL of this solution so obtained under the above operating conditions, 4-epidemethylchlortetracycline and demethylchlortetracycline are eluted in this order with the resolution between these peaks being not less than 3. The relative retention time of 4-epidemethylchlortetracycline with respect to demethylchlortetracycline is about 0.7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dibekacin Sulfate

硫酸ジベカシン

Change to read except the structural formula and chemical name:

Dibekacin Sulfate contains not less than 640 μg (potency) per mg, calculated on the dried basis. The potency of Dibekacin Sulfate is expressed as mass (potency) of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52).

Description Dibekacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 20 mg each of Dibekacin Sulfate and Dibekacin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28) and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same Rf value.

(2) To 5 mL of a solution of Dibekacin Sulfate (1 in 50) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation $[\alpha]_D^{20}$: +96 – +106° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Dibekacin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Dibekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying Not more than 5.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 6.5 to 6.6 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Dibekacin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Dibekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Diclofenamide

ジクロフェナミド

Change the Purity (4) to read:

Purity

(4) Related substances—Dissolve 0.10 g of Diclofenamide in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of diclofenamide from the sample solution is not larger than the peak area of diclofenamide from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of diclofenamide.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of diclofenamide obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of diclofenamide obtained from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diclofenamide is not more than 1.0%.

Change the Assay to read:

Assay Weigh accurately about 50 mg each of Diclofenamide and Diclofenamide Reference Standard, previously dried, and dissolve each in 30 mL of the mobile phase. To each add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diclofenamide to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_6H_6Cl_2N_2O_4S_2 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Diclofenamide Reference Standard

Internal standard solution—A solution of butyl parahydroxy benzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of sodium phosphate TS and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of diclofenamide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, diclofenamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diclofenamide to that of the internal standard is not more than 1.0%.

Diethylcarbamazine Citrate Tablets

クエン酸ジエチルカルバマジン錠

Change the Assay to read:

Assay Weigh accurately and powder not less than 20 Diethylcarbamazine Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$), add 10 mL of water, shake well, add 5 mL of sodium hydroxide TS, then add exactly 20 mL of the internal standard solution, and shake vigorously for 10 minutes. Centrifuge, discard the aqueous layer, and use the chloroform layer as the sample solution. Separately, weigh accurately about 50 mg of Diethylcarbamazine Citrate Reference Standard, previously dried at 105°C for 4 hours, dissolve in 10 mL of water, add 5 mL of sodium hydroxide TS, proceed in the same manner as the preparation of the sample solution, and use the chloroform layer as the standard solution. Perform the test with 2 μL of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diethycarbamazine to that of the internal standard, respectively.

$$\text{Amount (mg) of diethylcarbamazine citrate } (C_{10}H_{21}N_3O.C_6H_8O_7) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Diethylcarbamazine Citrate Reference Standard

Internal standard solution—A solution of *n*-octadecane in chloroform (1 in 1250).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube 3 mm in inside diameter and 1 m in length, packed with silanized siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with 35% methylphenyldimethyl silicone polymer for gas chromatography in the ratio of 3%.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of diethylcarbamazine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, diethylcarbamazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diethylcarbamazine to that of the internal standard is not more than 1.5%.

1% Dihydrocodeine Phosphate Powder

リン酸ジヒドロコデイン散 1%

Change the Assay to read:

Assay Weigh accurately about 5 g of 1% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay, separately determined its loss on drying (105°C, 4 hours), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard.

$$\text{Amount (mg) of dihydrocodeine phosphate } (C_{18}H_{23}NO_3.H_3PO_4) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

Internal standard solution—A solution of ethylefurin hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

10% Dihydrocodeine Phosphate Powder

リン酸ジヒドロコデイン散 10%

Change the Assay to read:

Assay Weigh accurately about 2.5 g of 10% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay, separately determined its loss on drying (105°C, 4 hours), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dihydrocodeine to that of the internal standard.

Amount (mg) of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)

$$= W_S \times \frac{Q_T}{Q_S} \times 5$$

W_S: Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

Internal standard solution—A solution of ethylefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Dihydroergotoxine Mesilate

メシル酸ジヒドロエルゴトキシン

Change the Assay (1) to read:

Assay (1) Dihydroergotoxine mesilate—Weigh accurately about 30 mg each of Dihydroergotoxine Mesilate and Dihydroergotoxine Mesilate Reference Standard, and dissolve them separately in a suitable amount of a mixture of water and acetonitrile (3:1). To these solutions add exactly 10 mL of the internal standard solution and an amount of a mixture of water and acetonitrile (3:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios of the peak areas of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to the peak area of the internal standard of these solutions.

Amount (mg) of dihydroergotoxine mesilate

$$= W_S \times \frac{M_{TA} + M_{TB} + M_{TC} + M_{TD}}{M_{SA} + M_{SB} + M_{SC} + M_{SD}}$$

W_S: Amount (mg) of Dihydroergotoxine Mesilate Reference Standard, calculated on the anhydrous basis

M_{TA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the sample solution × 659.80

M_{TB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the sample × 673.83

M_{TC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the sample solution × 707.85

M_{TD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the sample solution × 673.83

M_{SA}: Ratio of the peak area of dihydroergocornine to that of the internal standard of the standard solution × 659.80

M_{SB}: Ratio of the peak area of dihydro-α-ergocryptine to that of the internal standard of the standard solution × 673.83

M_{SC}: Ratio of the peak area of dihydroergocristine to that of the internal standard of the standard solution × 707.85

M_{SD}: Ratio of the peak area of dihydro-β-ergocryptine to that of the internal standard of the standard solution × 673.83

Internal standard solution—Dissolve 0.04 g of chloramphenicol in a mixture of water and acetonitrile (3:1) to make 250 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and triethylamine (30:10:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard, dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine are eluted in this order with the resolution between the peaks of dihydro-α-ergocryptine and dihydroergocristine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydroergocornine, dihydro-α-ergocryptine, dihydroergocristine and dihydro-β-ergocryptine to that of the internal standard is not more than 0.5%.

Diltiazem Hydrochloride

塩酸ジルチアゼム

Change the Purity (5) to read:

Purity

(5) Related substances—Dissolve 50 mg of Diltiazem Hydrochloride in 50 mL of diluted ethanol (99.5) (4 in 5), and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add diluted ethanol (99.5) (4 in 5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total peak area of peaks other than the peak of diltiazem obtained from the sample solution is not more than 3/5 times the peak area of diltiazem obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of *d*-camphorsulfonic acid in 500 mL of water, and filter using a membrane filter (0.4 μm in pore size). Add 250 mL each of acetonitrile and methanol to the filtrate, and adjust the solution to a pH of 6.6 by adding sodium acetate trihydrate.

Flow rate: Adjust the flow rate so that the retention time of diltiazem is about 9 minutes.

Time span of measurement: About twice as long as the retention time of diltiazem after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add diluted ethanol (99.5) (4 in 5) to make exactly 10 mL. Confirm that the peak area of diltiazem obtained from 20 μL of this solution is equivalent to 15 to 25% of that of diltiazem obtained from 20 μL of the standard solution.

System performance: Dissolve 0.03 g of Diltiazem Hydrochloride, 0.02 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1, 5-benzothiazepin-4-(5*H*)-one hydrochloride and 0.02 g of phenylbenzoate in 160 mL of ethanol (99.5), and add water to make 200 mL. Perform the test with 20 μL of this solution as directed under the Liquid Chromatography under the above operating conditions: *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl] - 2 - (4 - methoxyphenyl) - 1, 5 - benzothiazepin-4(5*H*)-one, diltiazem and phenyl benzoate are eluted in this order with the resolutions between the peaks of *d*-3-hydroxy-*cis* - 2, 3 - dihydro - 5 - [2 - (dimethylamino)ethyl] - 2 - (4 - methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one and diltiazem and between the peaks of diltiazem and phenyl benzoate being not less than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0%.

Dipyridamole

ジピリダモール

Change the Purity (4) to read:

Purity

(4) Related substances—Dissolve 50 mg of Dipyridamole in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of dipyridamole from the sample solution is not larger than the peak area of dipyridamole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

40°C.

Mobile phase: Dissolve 0.2 g of potassium dihydrogen phosphate in 200 mL of water, and add 800 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of dipyridamole is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of dipyridamole.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of dipyridamole obtained from 20 μL of this solution is equivalent to 15 to 25% of that of dipyridamole obtained from 20 μL of the standard solution.

System performance: Dissolve 7 mg of Dipyridamole and 3 mg of terphenyl in 50 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, dipyridamole and terphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dipyridamole is not more than 1.0%.

Dobutamine Hydrochloride

塩酸ドブタミン

Change the pH to read:

pH Dissolve 1.0 g of Dobutamine Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.5.

Change the Assay to read:

Assay Weigh accurately about 0.1 g each of Dobutamine Hydrochloride and Dobutamine Hydrochloride Reference Standard, each previously dried, dissolve separately in exactly 10 mL of the internal standard solution, add diluted methanol (1 in 2) to make 50 mL, and use these solutions as the sample solution and the standard solution respectively. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dobutamine to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{18}H_{23}NO_3.HCl = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Dobutamine Hydrochloride Reference Standard

Internal standard solution—A solution of salicylamide in diluted methanol (1 in 2) (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of tartrate buffer solution, pH 3.0 and methanol (7:3).

Flow rate: Adjust the flow rate so that the retention time of dobutamine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, dobutamine and internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dobutamine to that of the internal standard is not more than 1.0%.

Dopamine Hydrochloride Injection

塩酸ドパミン注射液

Change the Assay to read:

Assay To an exact volume of Dopamine Hydrochloride Injection, equivalent to about 30 mg of dopamine hydrochloride ($C_8H_{11}NO_2.HCl$), add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of dopamine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dopamine to that of the internal standard.

$$\text{Amount (mg) of dopamine hydrochloride } (C_8H_{11}NO_2.HCl) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of dopamine hydrochloride for assay

Internal standard solution—A solution of uracil in the mobile phase (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0

Flow rate: Adjust the flow rate so that the retention time of dopamine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and dopamine are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of dopamine to that of the internal standard is not more than 1.0%.

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Doxorubicin Hydrochloride

塩酸ドキソルビシン

Change to read except the structural formula and chemical name:

Doxorubicin Hydrochloride contains not less than 980 μg (potency) per mg, calculated on the anhydrous basis. The potency of Doxorubicin Hydrochloride is expressed as mass (potency) of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$. HCl).

Description Doxorubicin Hydrochloride occurs as a red-orange crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Doxorubicin Hydrochloride in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxorubicin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxorubicin Hydrochloride as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Doxorubicin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxorubicin Hydrochloride (1 in 200) responds to the Qualitative Test (1) for chloride.

Optical rotation $[\alpha]_D^{20}$: +240 – +290° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 50 mg of Doxorubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 50 mg of Doxorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Related substances—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the sample solution is not more than 1/4 times the peak area of doxorubicin from the standard solution, and the total area of the peaks other than doxorubicin is not more than the peak area of doxorubicin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of doxorubicin.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of doxorubicin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 with respect to doxorubicin, and doxorubicin are eluted in

this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 2.0%.

Water Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in water to make exactly 25 mL. Pipet 5 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances at 495 nm, A_T and A_S, of the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount [}\mu\text{g (potency)] of } C_{27}H_{29}NO_{11}.HCl = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Doxorubicin Hydrochloride Reference Standard

Containers and storage Containers—Tight containers.

Doxycycline Hydrochloride

塩酸ドキシサイクリン

Change to read except the structural formula and chemical name:

Doxycycline Hydrochloride contains not less than 880 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of ethanol. The potency of Doxycycline Hydrochloride is expressed as mass (potency) of doxycycline ($C_{22}H_{24}N_2O_8$: 444.43).

Description Doxycycline Hydrochloride occurs as yellow to dark yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Doxycycline Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Doxycycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg of Doxycycline Hydrochloride in 10 mL of water, and add silver nitrate TS: a white turbidity is produced.

Absorbance $E^{1\%}_{1\,cm}$ (349 nm): 285 – 315 (10 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL).

Optical rotation $[\alpha]_D^{20}$: -105 – $-120°$ (0.25 g calculated on the anhydrous basis and correcred by the amount of ethanol, 0.01 mol/L hydrochloric acid-methanol TS, 25 mL, 100 mm). Determine within 5 minutes after the sample solution is prepared.

Purity (1) Heavy metals—Proceed with 1.0 g of Doxycycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 5.0 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substance—Dissolve 20 mg of Doxycycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 6-epidoxycycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 6-epidoxycycline hydrochloride stock solution. Separately, dissolve 20 mg of metacycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as metacycline hydrochloride stock solution. Pipet 2 mL each of 6-epidoxycycline hydrochloride stock solution and metacycline hydrochloride stock solution, add 0.01 mol/L hydrochloric acid TS to make exatly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of metacycline and 6-epidoxycycline obtained from the sample solution are not more than the peak areas of them obtained from the standard solution, respectively, and the areas of the two peaks, appeared between the solvent peak and metacycline and behind of doxycycline, obtained from the sample solution are not more than 1/4 of the peak area of 6-epidoxycycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Mix 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS, 117 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 500 mL. To 400 mL of this solution add 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 25), 60 g of *t*-butanol and 200 mL of water, adjust the pH to 8.0 with 2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 19 minutes.

Time span of measurement: About 2.4 times as long as the retention time of doxycycline after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak areas of 6-epidoxycycline and metacycline obtained from 20 μL of this solution are equivalent to 3.5 to 6.5% of them obtained from 20 μL of the standard solution, respectively.

System performance: To 8 mL of the sample solution, 3 mL of 6-epidoxycycline hydrochloride stock solution and 2 mL of metacycline hydrochloride stock solution add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, metacycline, 6-epidoxycycline and doxycycline are eluted in this order with the resolutions between the peaks, metacycline and 6-epidoxycycline, and 6-epidoxycycline and doxycycline, being not less than 1.3 and not less than 2.0, respectively, and the symmetry coefficient of the peak of doxycycline is not more than 1.3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of metacycline and 6-epidoxycycline are not more than 3.0% and not more than 2.0%, respectively.

Ethanol Weigh accurately about 0.1 g of Doxycycline Hydrochloride, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of ethanol (99.5), and add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard: the amount of ethanol is not less than 4.3% and not more than 6.0%.

$$\text{Amount (\%) of ethanol} = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of ethanol (99.5)
W_T: Amount (mg) of the sample

Internal standard solution—A solution of 1-propanol (1 in 2000).

Operating conditions—

Detector: An hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 1.5 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (0.0075 μm in average pore size, 500 – 600 m^2/g in specific surface area) (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 135°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water Not less than 1.4% and not more than 2.8% (0.6 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.3% (1 g).

Assay Weigh accurately an amount of Doxycycline Hydrochloride and Doxycycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of doxycycline.

$$\text{Amount [}\mu\text{g (potency)] of doxycycline } (C_{22}H_{24}N_2O_8) = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Doxycycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 7.0 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, add 553 mL of a mixture of methanol and *N*,*N*-dimethyl-*n*-octylamine (550:3), and adjust the pH to 8.0 with a solution of sodium hydroxide (43 in 200).

Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of doxycycline are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Droperidol

ドロペリドール

Change the Description to read:

Description Droperidol occurs as a white to light yellow powder.

It is freely soluble in acetic acid (100), soluble in dichloromethane, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

Change the Identification to read:

Identification **(1)** Put 30 mg of Droperidol in a brown volumetric flask, and dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Transfer 5 mL of the solution to a brown volumetric flask, and add 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droperidol, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Droperidol in acetone, evaporate the acetone, dry the residue in a desiccator (in vacuum, silica gel, 70°C) for 4 hours, and perform the test with the residue.

Delete the Melting point.

Delete the following Monographs:

Drostanolone Propionate

プロピオン酸ドロスタノロン

Drostanolone Propionate Injection

プロピオン酸ドロスタノロン注射液

Enflurane

エンフルラン

Change the Purity (4) to read:

Purity

(4) Related substances—Proceed the test with 5 μL of Enflurane as directed under the Gas chromatography according to the following conditions. Determine each peak area other than the peak of air which appears soon after injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of enflurane is about 3 minutes.

Time span of measurement: About three times as long as the retention time of enflurane.

System suitability—

Test for required detection: Pipet exactly 1 mL of enflurane add 2-propanol to make exactly 100 mL. To exactly 2 mL of this solution add 2-propanol to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution, and add 2-propanol to make exactly 10 mL. Confirm that the peak area of enflurane obtained from 5 μL of this solution is equivalent to 7 to 13% of that of enflurane obtained from 5 μL of the solution for system suitability test.

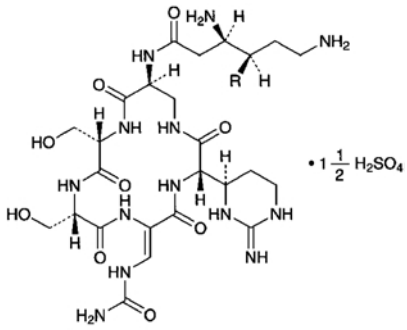
System performance: Mix 5 mL of Enflurane and 5 mL of 2-propanol. When the procedure is run with 5 μL of this mixture under the above operating conditions, enflurane and 2-propanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of enflurane is not more than 2.0%.

Add the following:

Enviomycin Sulfate

硫酸エンビオマイシン



Tuberactinomycin N : R = OH
Tuberactinomycin O : R = H

Tuberactinomycin N Sulfate

$C_{25}H_{43}N_{13}O_{10}.1\frac{1}{2}H_2SO_4$: 832.81

Tuberactinomycin O Sulfate

$C_{25}H_{43}N_{13}O_9.1\frac{1}{2}H_2SO_4$: 816.81

Tuberactinomycin N Sulfate
(3*R*,4*R*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diamino-4-hydroxyhexanamide sesquisulfate
[*33103-22-9*, Tuberactinomycin N]

Tuberactinomycin O Sulfate
(*S*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diaminohexanamide sesquisulfate
[*33137-73-4*, Tuberactinomycin O]

Enviomycin Sulfate contains not less than 770 μg (potency) per mg, calculated on the dried basis. The potency of Enviomycin Sulfate is expressed as mass (potency) of tuberactinomycin N ($C_{25}H_{43}N_{13}O_{10}$: 685.69).

Description Enviomycin Sulfate occurs as a white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Enviomycin Sulfate (1 in 200) add 1.5 mL of sodium hydroxide TS, and add 1 drop of a mixture of 0.01 mol/L citric acid TS and copper (II) sulfate TS (97:3) : a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Enviomycin Sulfate (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 2 mL of a solution of Enviomycin Sulfate (1 in 20) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation $[\alpha]_D^{20}$: -16 – $-22°$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 2.0 g of Enviomycin Sulfate in 20 mL of water is between 5.5 and 7.5.

Content ratio of the active principle Dissolve 0.1 g of Enviomycin Sulfate in water to make 100 mL, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_{T1} and A_{T2}, of tuberactinomycin N and tuberactinomycin O, having the relative retention time, 1.4 ± 0.4, with respect to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1} + A_{T2})$ is between 0.090 and 0.150.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate TS, 1,4-dioxane, tetrahydrofuran, water and ammonia solution (28) (100:75:50:23:2).

Flow rate: Adjust the flow rate so that the retention time of tuberactinomycin N is about 9 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the sample solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0%.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Enviomycin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying Not more than 4.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillius subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Enviomycin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

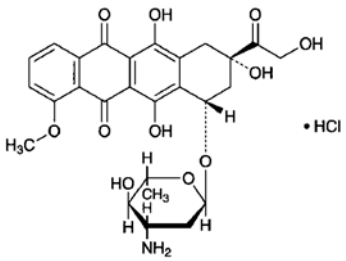
(4) Sample solutions—Weigh accurately an amount of Enviomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Add the following:

Epirubicin Hydrochloride

塩酸エピルビシン



$C_{27}H_{29}NO_{11}$.HCl: 579.98

(2*S*,4*S*)-4-(3-Amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyloxy)-1,2,3,4-tetrahydro-2,5,12-trihydroxy-2-hydroxyacetylnaphthacene-6,11-dione monohydrochloride [*56390-09-1*]

Epirubicin Hydrochloride contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvent. The potency of Epirubicin Hydrochloride is expressed as mass (potency) of epirubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl).

Description Epirubicin Hydrochloride occurs as a pale yellowish red to brownish red powder.

It is soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Epirubicin Hydrochloride in methanol (3 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Epirubicin Hydrochloride and Epirubicin Hydrochloride Reference Standard as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +310 – +340° (10 mg calculated on the anhydrous basis and collected by the amount of the residual solvent, methanol, 20 mL, 100 mm).

pH Dissolve 10 mg of Epirubicin Hydrochloride in 2 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution − Dissolve 50 mg of Epirubicin Hydrochloride in 5 mL of water: the solution is clear and dark red.

(2) Heavy metals—Proceed with 1.0 g of Epirubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the sum amount of the peaks other than epirubicin and 2-naphthalenesulfonic acid by the area percentage method: not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of epirubicin after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of epirubicin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(4) Residual solvents—Weigh accurately about 0.3 g of Epirubicin Hydrochloride, add exactly 0.6 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 6 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, add *N*,*N*-dimethylformamide to make exactly 25 mL, and use this solution as

methanol standard stock solution. Take exactly 125 μL of acetone, 30 μL of ethanol (99.5), 32 μL of 1-propanol and 17 μL of the methanol standard stock solution, add exactly 10 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following condition, and determine the ratios of the peak areas of acetone, ethanol, 1-propanol and methanol to that of the internal standard, Q_{TA} and Q_{SA}, Q_{TB} and Q_{SB}, Q_{TC} and Q_{SC}, and Q_{TD} and Q_{SD}, respectively. Calculate the amounts of acetone, ethanol, 1-propanol and methanol by the following equations: the amounts of acetone, ethanol, 1-propanol and methanol are not more than 1.5%, not more than 0.5%, not more than 0.5% and not more than 0.1%, respectively.

$$\text{Amount (\%) of acetone} = \frac{1}{W_T} \times \frac{Q_{TA}}{Q_{SA}} \times 593$$

$$\text{Amount (\%) of ethanol} = \frac{1}{W_T} \times \frac{Q_{TB}}{Q_{SB}} \times 142$$

$$\text{Amount (\%) of 1-propanol} = \frac{1}{W_T} \times \frac{Q_{TC}}{Q_{SC}} \times 154$$

$$\text{Amount (\%) of methanol} = \frac{1}{W_T} \times \frac{Q_{TD}}{Q_{SD}} \times 2.23$$

W_T: Amount (mg) of Epirubicin Hydrochloride

Internal standard solution—A solution of 1,4-dioxane in *N*,*N*-dimethylformamide (1 in 100).

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 30 m in length, coated with polyethylene glycol for gas-chromatography 1 μm in thickness.

Column temperature: 40°C for 11 minutes after injection of the sample, then rise to 90°C at a rate of 10°C per minute. If necessary, rise to 130°C at a rate of 50°C per minute and maintain the temperature for 30 minutes.

Injection port temperature: A constant temperature of about 120°C.

Detector temperature: A constant temperature of about 150°C.

Carrier gas: Herium

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 8 minutes.

Split ratio: 1:15

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone, methanol, ethanol, 1-propanol and the internal standard are eluted in this order with the resolution between the peaks of acetone and the internal standard being not less than 30.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of acetone, methanol, ethanol and 1-propanol are not more than 4.0%, respectively.

Water Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.5% (0.1 g).

Assay Weigh accurately an amount of Epirubicin Hydrochloride and Epirubicin Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of epirubicin to that of the internal standard.

$$\text{Amount [μg (potency)] of } C_{27}H_{29}NO_{11}.HCl = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Epirubicin Hydrochloride Reference Standard

Internal standard solution—A solution of sodium 2-naphthalene sulfonate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epirubicin is about 9.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature between 0°C and 5°C.

Ergocalciferol

エルゴカルシフェロール

Change the Assay to read:

Assay Weigh accurately about 30 mg each of Ergocalciferol and Ergocalciferol Reference Standard, and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 to 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ergocalciferol to that of the internal standard. Perform the procedure rapidly avoiding contact with air or other oxidizing agents and using light-resistant containers.

$$\text{Amount (mg) of } C_{28}H_{44}O = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Ergocalciferol Reference Standard

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with a silica gel for liquid chromatography (10 μm particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of hexane and n-amylalcohol (997:3).

Flow rate: Adjust the flow rate so that the retention time of ergocalciferol is about 25 minutes.

System suitability—

System performance: Dissolve 15 mg of Ergocalciferol Reference Standard in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil bath under a reflux condenser for 2 hours, and cool immediately to room temperature. Transfer the solution to a quartz test tube, and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To 10 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D_2, trans-vitamin D_2 and tachysterol$_2$ to that of ergocalciferol being about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D_2 and trans-vitamin D_2 being not less than 0.7, and that between ergocalciferol and tachysterol$_2$ being not less than 1.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ergocalciferol to that of the internal standard is not more than 1.0%.

Erythromycin

エリスロマイシン

Change to read except the structural formula and chemical name:

Erythromycin contains not less than 930 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$).

Description Erythromycin occurs as a white to light yellowish white powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in methanol and in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the infrared absorption spectrum of Erythromycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Erythromycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.01 g each of Erythromycin and Erythromycin Reference Standard in 1 mL of methanol, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 100°C for 15 minutes: the principal spot from the sample solution and the spot from the standard solution are dark purple in color, and their *R*f values are the same.

Optical rotation $[\alpha]_D^{20}$: -71 – $-78°$ (1 g calculated on the anhydrous basis, ethanol (95), 50 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Erythromycin according to Method 5 using hydrochloric acid instead of diluted hydrochloric acid (1 in 2), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 10 mL, and use this solution as the sample solution.

Separately, dissolve 16 mg of Erythromycin Reference Standard in 2 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the sample solution are not more than those of erythromycin B and erythromycin C from the standard solution, respectively, and each area of the peaks other than erythromycin, erythromycin B and erythromycin C is not more than the area of the peak of erythromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 190 mL of *t*-butanol, 30 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of erythromycin is about 20 minutes.

Time span of measurement: About 4 times as long as the retention time of erythromycin after the solvent peak.

System suitability—

System performance: Dissolve 2 mg of *N*-demethylerythromycin in 10 mL of the standard solution. When the procedure is run with 100 μL of this solution under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin and erythromycin B are eluted in this order, with the resolution between the peaks of *N*-demethylerythromycin and erythromycin C being not less than 0.8, and with the resolution between the peaks of *N*-demethylerythromycin and erythromycin being not less than 5.5.

System repeatability: When the test is repeated 3 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of erythromycin is not more than 3.0%.

Water Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

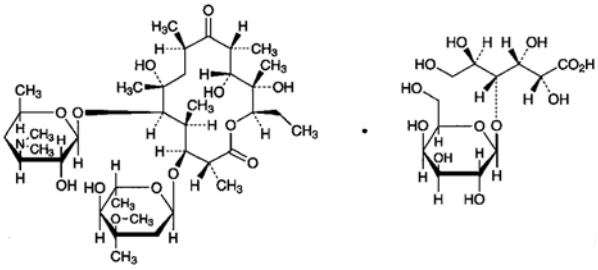
(4) Sample solutions—Weigh accurately an amount of Erythromycin, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Add the following:

Erythromycin Lactobionate

ラクトビオン酸エリスロマイシン



$C_{37}H_{67}NO_{13}.C_{12}H_{22}O_{12}$: 1092.22

(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide mono(4-*O*-β-D-galactopyranosyl-D-gluconate) [*3847-29-8*]

Erythromycin Lactobionate contains not less than 590 μg (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Lactobionate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Lactobionate occurs as a white powder.

It is freely soluble in water, in methanol and in ethanol (99.5), and very slightly soluble in acetone.

Identification **(1)** To 3 mg of Erythromycin Lactobionate add 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color is produced, and it changes immediately to red to deep purple.

(2) Transfer about 0.3 g of Erythromycin Lactobionate to a separator, add 15 mL of ammonia TS and 15 mL of chloroform, shake, and take the separated aqueous layer. Wash the aqueous layer with three 15-mL portions of chloroform, and evaporate the aqueous liquid on a water bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:2), and use this solution as the sample solution. Separately, dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatograph. Develop the plate with the upper layer obtained from a mixture of water, 1-butanol and acetic acid (100) (3:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid, and heat at 105°C for 20 minutes: the principal spot obtained from the sample solution shows a deep brown and the *R*f value which are the same as those of the principal spot from the standard solution.

pH The pH of a solution obtained by dissolving 0.5 g of Erythromycin Lactobionate in 10 mL of water is between 5.0 and 7.5.

Water Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Erythromycin Lactobionate, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Estradiol Benzoate

安息香酸エストラジオール

Change the Assay to read:

Assay Weigh accurately about 10 mg each of Estradiol Benzoate and Estradiol Benzoate Reference Standard, previously dried, and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, add 5 mL of the internal standard solution, then add methanol to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of estradiol benzoate to that of the internal standard.

$$\text{Amount (mg) of } C_{25}H_{28}O_3 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Estradiol Benzoate Reference Standard

Internal standard solution—A solution of progesterone in methanol (13 in 80,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of estradiol benzoate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and estradiol benzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estradiol benzoate to that of the internal standard is not more than 1.0%.

Estriol

エストリオール

Change the Assay to read:

Assay Weigh accurately about 25 mg each of Estriol and Estriol Reference Standard, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of estriol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{18}H_{24}O_3 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Estriol Reference Standard

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (51:49).

Flow rate: Adjust the flow rate so that the retention time of estriol is about 10 minutes.

System suitability—

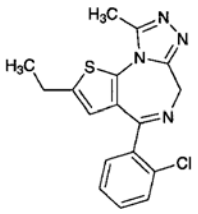
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, estriol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estriol to that of the internal standard is not more than 1.0%.

Add the following:

Etizolam

エチゾラム



$C_{17}H_{15}ClN_4S$: 342.85

4-(2-Chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine

[*40054-69-1*]

Etizolam contains not less than 98.5% and not more than 101.0% of etizolam ($C_{17}H_{15}ClN_4S$).

Description Etizolam occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), sparingly soluble in acetonitrile and in acetic anhydride, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Etizolam in ethanol (99.5) (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etizolam as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit

similar intensities of absorption at the same wave numbers.

Melting point 146 – 149°C

Purity **(1)** Heavy metals—Proceed with 2.0 g of Etizolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Etizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 20 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etizolam obtained from the sample solution is not more than the peak area of etizolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with potassium hydroxide TS. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of etizolam after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of etizolam obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the standard solution.

System performance: Dissolve 0.02 g each of Etizolam and ethyl parahydroxybenzoate in the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, etizolam and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2%.

Loss on drying Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.3 g of Etizolam, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.14 mg of $C_{17}H_{15}ClN_4S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Famotidine for Injection

注射用ファモチジン

Change the Purity (2) to read:

Purity

(2) Related substances—Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the sample solition. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than peak of famotidine from the sample solution is not larger than peak area of famotidine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of famotidine after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the water to make exactly 20 mL. Confirm that the peak area of famotidine obtained from 5 μL of this solution is equivalent to 8 to 12% of that of famotidine obtained from 5 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

Delete the following Monograph:

Floctafenine

フロクタフェニン

Flomoxef Sodium

フロモキセフナトリウム

Change to read except the structural formula and chemical name:

Flomoxef Sodium contains not less than 862 μg (potency) per mg, calculated on the anhydrous basis. The potency of Flomoxef Sodium is expressed as mass (potency) of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Description Flomoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (99.5).

Identification **(1)** Decompose 0.01 g of Flomoxef Sodium as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. To 2 mL of the test solution so obtained add 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1): blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Flomoxef Sodium (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flomoxef Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the spectrum of a solution of Flomoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 100) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ3.5 ppm, a single signal or a sharp multiple signal B at around δ3.7 ppm, and a single signal C at around δ5.2 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:1.

(5) Flomoxef Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: -8 – $-13°$ (1 g calculated on the anhydrous basis, a mixture of water and ethanol (99.5) (4:1), 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.5 g of Flomoxef Sodium in 5 mL of water is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Flomoxef Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Flomoxef Sodium in a quartz crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—To 1.0 g of Flomoxef Sodium 5 mL of sulfuric acid and 5 mL of nitric acid, heat carefully until the solution changes to colorless to light yellow with occasional addition of 2 mL of nitric acid. After cooling, add 10 mL of ammonium oxalate TS, heat until white fumes evolve, and concentrate to 2 to 3 mL. After cooling, add water to make 10 mL, and perform the test using this solution as the test solution: the color is not darker than that of the control solution.

Control solution: Proceed to prepare a solution in the same manner as the test solution without Flomoxef Sodium, and transfer 10 mL of the solution so obtained to the generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test solution (not more than 2 ppm).

(4) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution obtained in the Assay and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard: the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol is not more than 1.0% of the amount of Flomoxef Sodium calculated on the anhydrous basis.

Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol ($C_3H_6N_4OS$)

$$= W_S \times \frac{Q_T}{Q_S} \times \frac{1}{10}$$

W_S: Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Water Not more than 1.5% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Flomoxef Sodium and Flomoxef Triethylammonium Reference Standard,

equivalent to about 50 mg (potency), and dissolve each in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of flomoxef to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of flomoxef } (C_{15}H_{18}F_2N_6O_7S_2) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Flomoxef Triethylammonium Reference Standard

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 246 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 – 10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate 12-water and 1.60 g of tetra-*n*-butylammonium bromide in water to make 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of flomoxef is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, flomoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of flomoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At 5°C or below.

Flurbiprofen

フルルビプロフェン

Change the Purity (3) to read:

Purity

(3) Related substances—Dissolve 20 mg of Flurbiprofen in 10 mL of a mixture of water and acetonitrile (11:9), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (11:9) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks other than the peak of flurbiprofen from the sample solution is not larger than the peak area of flurbiprofen from the standard solution, and the total area of these peaks is not larger than twice the peak area of flurbiprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (12:7:1).

Flow rate: Adjust the flow rate so that the retention time of flurbiprofen is about 20 minutes.

Time span of measurement: About twice as long as the retention time of flurbiprofen after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add a mixture of water and acetonitrile (11:9) to make exactly 25 mL. Confirm that the peak area of flurbiprofen obtained from 20 μL of this solution is equivalent to 16 to 24% of that of flurbiprofen obtained from 20 μL of the standard solution.

System performance: Dissolve 0.04 g of flurbiprofen and 0.02 g of butyl parahydroxybenzoate in 100 mL of a mixture of water and acetonitrile (11:9). To 5 mL of this solution add a mixture of water and acetonitrile (11:9) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and flurbiprofen are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flurbiprofen is not more than 2.0%.

Folic Acid

葉酸

Change the Water to read:

Water Not more than 8.5% (10 mg, coulometric titration).

Fradiomycin Sulfate

硫酸フラジオマイシン

Change to read except the structural formula and chemical name:

Fradiomycin Sulfate, when dried, contains not less than 623 μg (potency) per mg. The potency of Fradiomycin Sulfate is expressed as mass (potency) of fradiomycin ($C_{23}H_{46}N_6O_{13}$: 614.64).

Description Fradiomycin Sulfate occurs as a white to light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Dissolve 0.05 g each of Fradiomycin Sulfate and Fradiomycin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the *R*f values of the principal spots from the sample solution and the standard solution are not different each other.

(2) A solution of Fradiomycin Sulfate (1 in 20) responds to the Qualitative Test (1) for sulfate.

Optical rotation $[\alpha]_D^{20}$: +53.5 – +59.0° (1 g calculated on the dried basis, water, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Fradiomycin Sulfate in 10 mL of water is between 5.0 and 7.5.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Fradiomycin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Fradiomycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.63 g of Fradiomycin Sulfate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the spot at around *R*f 0.4 from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 8.0% (0.2 g, in vaccum, 60°C, 3 hours).

Residue on ignition Not more than 0.30% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Agar medium for seed and base layer

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients and sterilize. Adjust the pH after sterilization to 7.8 – 8.0 with sodium hydroxide TS.

(3) Standard solutions – Weigh accurately an amount of Fradiomycin Sulfate Reference Standard, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Fradiomycin Sulfate, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Gentamicin Sulfate

硫酸ゲンタマイシン

Change to read except the structural formula and chemical name:

Gentamicin Sulfate contains not less than 590 μg (potency) per mg, calculated on the dried basis. The potency of Gentamicin Sulfate is expressed as mass (potency) of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Description Gentamicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 50 mg of Gentamicin Sulfate in 1 mL of water, and add 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 500). Gently superimpose this solution on 1 mL of sulfuric acid: a blue-purple color develops at the zone of contact.

(2) Dissolve 50 mg each of Gentamicin Sulfate and Gentamicin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm^2, and without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapors: three principal spots obtained from the sample solution are the same with the corresponding spots from the standard solution in color tone and the *R*f value, respectively.

(3) Dissolve 50 mg of Gentamicin Sulfate in 5 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{25}$: +107 – +121° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.20 g of Gentamicin Sulfate in 5 mL of water is between 3.5 and 5.5.

Content ratio of the active principle Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm^2, without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor. Determine the integral absorbances, A_a, A_b and A_c, of the colored spots of gentamicin C_1 (*R*f value: about 0.3), gentamicin C_2 (*R*f value: about 0.2) and gentamicin C_{1a} (*R*f value: about 0.1), respectively, by a densitometer (wavelength: 450 nm) while covering the plate with a glass plate, and calculate these amounts by the following formulae: gentamicin C_1 is between 25% and 55%, gentamicin C_2 is between 25% and 50%, and gentamicin C_{1a} is between 5% and 30%.

$$\text{Amount (\%) of gentamicin } C_1 = \frac{A_a}{A_a + 1.35A_b + A_c} \times 100$$

$$\text{Amount (\%) of gentamicin } C_2 = \frac{1.35A_b}{A_a + 1.35A_b + A_c} \times 100$$

$$\text{Amount (\%) of gentamicin } C_{1a} = \frac{A_c}{A_a + 1.35A_b + A_c} \times 100$$

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Gentamicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 2.0 g of Gentamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm^2, without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor, and compare the colored spots while covering with a glass plate: the spots other than the spots of gentamicin C_1 (*R*f value: about 0.3), gentamicin C_2 (*R*f value: about 0.2) and gentamicin C_{1a} (*R*f value: about 0.1) obtained from the sample solution are not more intense than the spot of gentamicin C_2 from the standard solution.

Loss on drying Not more than 18.0% (0.15 g, reduced pressure not exceeding 0.67 kPa, 110°C, 3 hours). Handle the sample avoiding absorption of moisture.

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate

method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus epidermidis* ATCC 12228

(2) Agar media for seed and base layer—

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(3) Agar medium for transferring test organisms—Use the medium ii in 2) Medium for other organisms under (2) Agar media for transferring test organisms.

(4) Standard solutions—Weigh accurately an amount of Gentamicin Sulfate Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 15°C or lower, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(5) Sample solutions—Weigh accurately an amount of Gentamicin Sulfate, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Glycerin

グリセリン

Change the origin/limits of content to read:

Glycerin contains not less than 84.0% and not more than 87.0% of $C_3H_8O_3$.

Change the Description to read:

Description Glycerin is a clear, colorless, viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Change the Identification to read:

Identification Determine the infrared absorption spectrum of Glycerin as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Add the following next to Purity:

Water 13 – 17% (0.1 g, volumetric titration, direct titration).

Add the following next to Residue on ignition:

Assay Weigh accurately about 0.2 g of Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.209 mg of $C_3H_8O_3$

Concentrated Glycerin

濃グリセリン

Change the origin/limits of content to read:

Concentrated Glycerin contains not less than 98.0% and not more than 101.0% of glycerin ($C_3H_8O_3$), calculated of the anhydrous basis.

Change the Description to read:

Description Concentrated Glycerin is a clear, colorless and viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Change the Identification to read:

Identification Determine the infrared absorption spectrum of Concentrated Glycerin as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Add the following next to Purity:

Water Not more than 2.0% (6 g, volumetric titration, direct titration).

Add the following next to Residue on ignition:

Assay Weigh accurately about 0.2 g of Concentrated Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.209 mg of $C_3H_8O_3$

Add the following:

Gramicidin

グラミシジン

[*1405-97-6*]

Gramicidin is a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus brevis* Dubos.

It contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Gramicidin is expressed as mass (potency) of gramicidin.

Description Gramicidin occurs as a white to light yellowish white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** To 10 mg of Gramicidin add 2 mL of 6 mol/L hydrochloric acid TS, and heat in a water bath for 30 minutes with occasional stirring. After cooling, neutralize with 6 mol/L sodium hydroxide TS, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 2 minutes: a blue-purple to red-purple color develops.

(2) Determine the absorption spectrum of a solution of Gramicidin in ethanol (95) (1 in 20,000), as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gramicidin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Loss on drying Not more than 3.0% (0.1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Turbidimetric method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Enterococcus hirae* ATCC 10541

(2) Agar medium for transferring test organism

Glucose	10.0 g
Casein peptone	5.0 g
Yeast extract	20.0 g
Potassium dihydrogen phosphate	2.0 g
Polysorbate 80	0.1 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.7 to 6.8 after sterilization.

(3) Liquid medium for suspending test organism—Use the culture medium (2).

(4) Preparation of the test organism suspension—Puncture the test organism in the medium, prepared by dispensing 10 mL of the agar medium for transferring test organism in a test tube about 16 mm in inside diameter, incubate at 36.5 to 37.5°C for 20 to 24 hours. After sub-culturing at least three times, keep between 1 to 5°C. Transfer the organism so obtained in 10 mL of the liquid medium for suspending test organism, incubate at 36.5 to 37.5°C for 20 to 24 hours, and use this medium as the test organism stock suspension. Before use, add the test organism stock suspension to the liquid medium for suspending test organism so that the transmittance at 580 nm is 50 to 60%. Mix one volume of this suspension and 200 volume of the liquid medium for suspending test organism, and use this as the test organism suspension.

(5) Standard solution—Weigh accurately an amount of Gramicidin Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 3 hours, equivalent to about 10 mg (potency), dissolve in ethanol (99.5) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add the following diluting solution to make a solution so that each mL contains 0.02 μg (potency), and use this solution as the standard solution.

Diluting solution: To 390 mL of propylene glycol add 210 mL of a mixture of ethanol (99.5) and acetone (9:1) and Sterile Purified Water to make 1000 mL.

(6) Sample solution—Weigh accurately an amount of Gramicidin, equivalent to about 10 mg (potency), and dissolve in ethanol (99.5) to make exactly 100 mL. Take exactly a suitable amount of this solution, add the diluting solution obtained in (5) to make a solution so that each mL contains 0.02 μg (potency), and use this solution as the sample solution.

(7) Procedure—Transfer 0.155 mL, 0.125 mL, 0.100 mL, 0.080 mL and 0.065 mL each of the standard solution, 0.100 mL of the sample solution and 0.100 mL of the diluting solution, separately, in test tubes about 14 mm in inside diameter and about 15 cm in length, and make three sets for each. To each of the test tube add 10 mL of the test organism suspension, stopper the tube, incubate in a water bath at 36.5 to 37.5°C for 180 to 270 minutes, add 0.5 mL of a solution of formaldehyde (1 in 3), and determine their transmittances at 580 nm.

Containers and storage Containers—Tight containers.

Griseofulvin

グリセオフルビン

Change to read except the structural formula and chemical name:

Griseofulvin contains not less than 960 μg (potency) per mg, calculated on the dried basis. The potency of Griseofulvin is expressed as mass (potency) of griseofulvin ($C_{17}H_{17}ClO_6$).

Description Griseofulvin occurs as white, crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Griseofulvin in ethanol (95) (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Griseofulvin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Griseofulvin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Griseofulvin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +350 – +364° (0.25 g calculated on the dried basis, *N*,*N*-dimethylformamide, 25 mL, 100 mm).

Melting point 218 – 222°C

Purity **(1)** Acid—Dissolve 0.25 g of Griseofulvin in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.02 mol/L sodium hydroxide VS: the color of the solution is red.

(2) Heavy metals—Proceed with 1.0 g of Griseofulvin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Griseofulvin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Griseofulvin add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the sample solution. Separately, to 5.0 mg of Griseofulvin Reference Standard add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratio, Q_1, of the peak area of dechlorogriseofulvin, having the relative retention time of about 0.6 with respect to griseofulvin, to that of the internal standard obtained from the sample solution, the ratio, Q_2, of the peak area of dehydrogriseofulvin, having the relative retention time of about 1.2 with respect to griseofulvin, to that of the internal standard obtained from the sample solution and the ratio, Q_S, of the peak area of griseofulvin to that of the internal standard obtained from the standard solution: Q_1/Q_S is not more than 0.6, and Q_2/Q_S is not more than 0.15.

Internal standard solution—A solution of 9,10-diphenylanthracene in acetone (1 in 500).

Operating conditions—

Detector: An hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated with 25% phenyl-25% cyanopropylmethylsilicone polymer for gas chromatography in the ratio of 1% (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 250°C.

Temperature of injection port: A constant temperature of about 270°C.

Temperature of detector: A constant temperature of about 300°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of griseofulvin is about 10 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the internal standard solution diluted with acetone (1 in 10) to make exactly 10 mL. Confirm that the ratio of the peak area of griseofulvin to that of the internal standard obtained from 2 μL of this solution is equivalent to 7 to 13% of that obtained from 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and griseofulvin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of griseofulvin to that of the internal standard is not more than 5.0%.

(5) Petroleum ether soluble substances—To 1.0 g of Griseofulvin add 20 mL of petroleum ether, shake, and boil for 10 minutes under a reflux condenser. After cooling, filter through a dried filter paper, wash the filter paper with two 15-mL portions of petroleum ether, combine the washings to the filtrate, evaporate the petroleum ether on a water bath, and dry the residue at 105°C for 1 hour: the amount of the residue is not more than 0.2%.

Loss on drying Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 0.20% (1 g).

Assay Weigh accurately an amount of Griseofulvin and Griseofulvin Reference Standard, equivalent to about 50 mg (potency), dissolve each in 50 mL of *N*,*N*-dimethylformamide, add exactly 20 mL of the internal standard solution and water to make 250 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of griseofulvin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{17}H_{17}ClO_6 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Griseofulvin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of griseofulvin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, griseofulvin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of griseofulvin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Haloxazolam

ハロキサゾラム

Change the Purity (5) to read:

Purity

(5) Related substances—Dissolve 0.10 g of Haloxazolam in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the haloxazolam from the sample solution is not larger than the peak area of the haloxazolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.2 g of boric acid and 7.5 g of potassium chloride in 900 mL of water, adjust the pH with triethylamine to 8.5, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of haloxazolam is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of haloxazolam after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of haloxazolam obtained from 10 μL of this solution is equivalent to 8 to 12% of that of haloxazolam obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, haloxazolam and cloxazolam are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloxazolam is not more than 1.0%.

Homochlorcyclizine Hydrochloride

塩酸ホモクロルシクリジン

Change the Description to read:

Description Homochlorcyclizine Hydrochloride occurs as white to pale brown, crystals or powder.

It is very soluble in water, freely soluble in acetic acid (100), slightly soluble in ethanol (99.5), and very slightly soluble in acetonitrile and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

It is colored slightly by light.

A solution of Homochlorcyclizine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 227°C (with decomposition).

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Homochlorcyclizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Homochlorcyclizine Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Homochlorcyclizine Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Change the Purity to read:

Purity **(1)** Heavy metals—Proceed with 1.0 g of Homochlorcyclizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Homochlorcyclizine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Measure exactly 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas by the automatic integration method: the areas of the peaks other than homochlorcyclizine obtained from the sample solution are not more than 1/2 times the peak area of homochlorcyclizine from the standard solution, and the total area of the peaks other than homochlorcyclizine from the sample solution is not more than the peak area of homochlorcyclizine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 223 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (134:66:1).

Flow rate: Adjust the flow rate so that the retention time of homochlorcyclizine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of homochlorcyclizine.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of homochlorcyclizine obtained from 10 μL of this solution is equivalent to 7 to 13% of that of homochlorcyclizine obtained from 10 μL of the standard solution.

System performance: Dissolve 5 mg each of Homochlorcyclizine Hydrochloride and methyl parahydroxybenzoic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoic acid and homochlorcyclizine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of homochlorcyclizine is not more than 1.0%.

Hydrochlorothiazide

ヒドロクロロチアジド

Change the Assay to read:

Assay Weigh accurately about 30 mg each of Hydrochlorothiazide and Hydrochlorothiazide Reference Standard, previously dried, and dissolve in 150 mL of the mobile phase, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrochlorothiazide to that of the internal standard.

$$\text{Amount (mg) of } C_7H_8ClN_3O_4S_2 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Hydrochlorothiazide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in acetonitrile (9 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L sodium dihydrogen phosphate TS, pH 3.0 and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of hydrochlorothiazide is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrochlorothiazide and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

Hydrocortisone Acetate

酢酸ヒドロコルチゾン

Change the Assay to read:

Assay Dissolve about 20 mg each of Hydrocortisone Acetate and Hydrocortisone Acetate Reference Standard, previously dried and accurately weighed, in methanol, add exactly 10 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone acetate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{23}H_{32}O_6 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Hydrocortisone Acetate Reference Standard

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone acetate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone acetate to that of the internal standard is not more than 1.0%.

Hydrocortisone Sodium Phosphate

リン酸ヒドロコルチゾンナトリウム

Cange the Purity (6) to read:

Purity

(6) Free hydrocortisone—Dissolve 25 mg of Hydrocortisone Sodium Phosphate in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh 25 mg of Hydrocortisone Reference Standard, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of hydrocortisone from each solution: A_T is not larger than A_S.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrocortisone is not more than 1.0%.

Change the Assay to read:

Assay Weigh accurately about 20 mg each of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard (determine its loss on drying before using), dissolve each in 50 mL of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrocortisone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{29}Na_2O_8P = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Hydrocortisone Sodium Phosphate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS, pH 2.6 and methanol (1:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone phosphate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone phosphate and isopropyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone phosphate to that of the internal standard is not more than 1.0%.

Idarubicin Hydrochloride

塩酸イダルビシン

Change the Water to read:

Water Not more than 5.0% (0.1 g, coulometric titration).

Idoxuridine Ophthalmic Solution

イドクスウリジン点眼液

Change the Purity to read:

Purity 5-Iodouracil and 2′-deoxyuridine—To a volume of Idoxuridine Ophthalmic Solution, equivalent to 4.0 mg of Idoxuridine according to the labeled amount, add water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 12.0 mg of 5-iodouracil for liquid chromatography and 4.0 mg of 2′-deoxyuridine for liquid chromatography in water to make exactly 200 mL. Measure exactly 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas of 5-iodouracil and 2′-deoxyuridine: the peak areas of 5-iodouracil and 2′-deoxyuridine of the sample solution are not more than the peak areas of 5-iodouracil and 2′-deoxyuridine of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (24:1).

Flow rate: Adjust the flow rate so that the retention time of 2′-deoxyuridine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 2′-deoxyuridine and 5-iodouracil are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 2′-deoxyuridine is not more than 1.0%.

Change the Assay to read:

Assay Measure exactly a volume of Idoxuridine Ophthalmic Solution, equivalent to 3 mg of idoxuridine ($C_9H_{11}IN_2O_5$) according to the labeled amount, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Separately weigh accurately about 10 mg of Idoxuridine Reference Standard, previously dried at 60°C for 3 hours, dissolve in water to make exactly 10 mL. Measure exactly 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of idoxuridine to that of the internal standard, respectively.

$$\text{Amount (mg) of idoxuridine } (C_9H_{11}IN_2O_5) = W_S \times \frac{Q_T}{Q_S} \times \frac{3}{10}$$

W_S: Amount (mg) of Idoxuridine Reference Standard

Internal standard solution—A solution of sulfathiazole in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (87:13).

Flow rate: Adjust the flow rate so that the retention time of idoxuridine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, idoxuridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of idoxuridine to that of the internal standard is not more than 1.0%.

Imipenem

イミペネム

Change to read except the structural formula and chemical name:

Imipenem contains not less than 924 μg (potency) per mg, calculated on the anhydrous basis. The potency of Imipenem is expressed as mass (potency) of imipenem ($C_{12}H_{17}N_3O_4S$: 299.35).

Description Imipenem occurs as white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Imipenem in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipenem Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Imipenem as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Imipenem Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: +84 – +89° (50 mg calculated on the anhydrous basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Imipenem in 200 mL of water is between 4.5 and 7.0.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Imipenem according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Put 2.0 g of Imipenem in a crucible, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes evolve. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure once more. Then add 2 mL of hydrogen peroxide (30), heat, and repeat this procedure several times until the color of the solution changes to colorless to pale yellow. After cooling, heat again until white fumes evolve. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 1 ppm).

(3) Related substances—Dissolve 50 mg of Imipenem in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of thienamycin, having the relative retention time of about 0.8 with respect to imipenem, obtained from the sample solution is not more than the peak area of imipenem from the standard solution, the area of the peak other than imipenem and thienamycin from the sample solution is not more than 1/3 times the peak area of imipenem from the standard solution, and the total area of the peaks other than imipenem and thienamycin from the sample solution is not more than the peak area of imipenem from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of imipenem.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, add 0.1 mol/L 3-(*N*-morpholino)-propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL. Confirm that the peak area of imipenem from 10 μL of this solution is equivalent to 7 to 13% of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 2.0%.

Water Not less than 5.0% and not more than 8.0% (20 mg, coulometric titration, water evaporation temperature: 140°C).

Residue on ignition Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Imipenem and Imipenem Reference Standard, equivalent to about 50 mg (potency), dissolve each in 0.1 mol/L 3-(*N*-morpholino)-propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL

each of the sample solution and the standard solution, within 30 minutes after preparation of these solutions, as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of imipenem of these solutions.

$$\text{Amount [}\mu\text{g (potency)] of } C_{12}H_{17}N_3O_4S = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Imipenem Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 and acetonitrile (100:1).

Flow rate: Adjust the flow rate so that the retention time of imipenem is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg of Imipenem and 75 mg of resorcinol in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0. When the procedure is run with 10 μL of this solution under the above operating conditions, imipenem and resorcinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 0.80%.

Containers and storage Containers—Hermetic containers.

Indometacin Suppositories

インドメタシン坐剤

Change the Assay to read:

Assay Weigh accurately not less than 20 Indometacin Suppositories, cut into small pieces carefully, and mix well. Weigh accurately a portion of the mass, equivalent to about 50 mg of indometacin ($C_{19}H_{16}ClNO_4$), add 40 mL of tetrahydrofuran, warm at 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Filter the solution, discard the first 10 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 100 mL. Allow the solution to stand for 30 minutes, filter through a membrane filter (0.5 μm pore size), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Indometacin Reference Standard, previously dried at 105°C for 4 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of the solution, proceed in the same manner as the sample solution, and use as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of indometacin to that of the internal standard, respectively.

$$\text{Amount (mg) of indometacin } (C_{19}H_{16}ClNO_4) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Indometacin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7:3).

Flow rate: Adjust the flow rate so that the retention time of indometacin is about 8 minutes.

System suitability—

System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0 and between the peaks of parahydroxybenzoate and indometacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

Delete the following Monographs:

Iopanoic Acid

イオパノ酸

Iopanoic Acid Tablets

イオパノ酸錠

Isoniazid Injection

イソニアジド注射液

Change the Description to read:

Description Isoniazid Injection occurs as a clear, colorless liquid.

pH: 6.5 – 7.5.

Change the Identification to read:

Identification To a volume of Isoniazid Injection, equivalent to 20 mg of Isoniazid according to the labeled amount, and add water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 264 nm and 268 nm.

Change the Assay to read:

Assay To an exactly measured volume of Isoniazid Injection, equivalent to about 50 mg of isoniazid ($C_6H_7N_3O$), add water to make exactly 100 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of isoniazid to that of the internal standard.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of isoniazid for assay

Internal standard solution—A solution of propyl parahydroxybenzoate (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to make a solution having pH 2.5. To 500 mL of this solution add 500 mL of methanol, and add 2.86 g of sodium tridecanesulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, isoniazid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of isoniazid to that of the internal standard is not more than 1.3%.

Isoniazid Tablets

イソニアジド錠

Change the Assay to read:

Assay Weigh accurately and powder not less than 20 Isoniazid Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.10 g of isoniazid ($C_6H_7N_3O$), add 150 mL of water, shake for 30 minutes, then add water to make exactly 200 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of isoniazid of the sample solution and the standard solution.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = W_S \times \frac{A_T}{A_S} \times 2$$

W_S: Amount (mg) of isoniazid for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to adjust the pH to 2.5. To 400 mL of this solution add 600 mL of methanol, and dissolve 2.86 g of sodium tridecanesulfonate in this.

Flow rate: Adjust the flow rate so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: Dissolve 5 mg of Isoniazid and 5 mg of isonicotinic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid and isoniazid are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoniazid is not more than 1.0%.

Josamycin

ジョサマイシン

Change to read except the structural formula and chemical name:

Josamycin contains not less than 900 μg (potency) per mg, calculated on the dried basis. The potency of Josamycin is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$).

Description Josamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Josamycin in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin and Josamycin Reference Standard in 1 mL of methanol, add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions: the retention time of the main peak obtained from the sample solution is the same with that of the peak of josamycin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity (1) Heavy metals—Proceed with 1.0 g of Josamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin in 5 mL of methanol, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of josamycin and the related substances by the area percentage method: the amount of any peak other than josamycin is not more than 6%, and the total of these peaks is not more than 20%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 119 g of sodium perchlorate in water to make 1000 mL, and adjust the pH to 2.5 with 1 mol/L hydrochloric acid TS. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin is about 10 minutes.

Time span of measurement: About 4 times as long as the retention time of josamycin after the solvent peak.

System suitability—

Test for required detectability: Pipet 3 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add diluted methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of josamycin obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.05 g of Josamycin in 50 mL of 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0, and allow to stand at 40°C for 3 hours. Adjust the pH of this solution to 6.8 to 7.2 with 2 mol/L sodium hydroxide TS, and add 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of josamycin S_1, which relative retention time to josamycin is about 0.9, and josamycin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard devia-

tion of the peak area of josamycin is not more than 1.5%.

Loss on drying Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Josamycin Reference Standard, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add water to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Josamycin, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of this solution, add water to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Josamycin Propionate

プロピオン酸ジョサマイシン

Change to read except the structural formula and chemical name:

Josamycin Propionate contains not less than 843 μg (potency) per mg, calculated on the dried basis. The potency of Josamycin Propionate is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$).

Description Josamycin Propionate occurs as a white to light yellowish white crystalline powder.

It is very soluble in acetonitrile, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Josamycin Propionate in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Propionate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin Propionate and Josamycin Propionate Reference Standard in 50 mL of diluted acetonitrile (1 in 2), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions: the retention time of the peak of josamycin propionate obtained from the sample solution is the same with that of the peak of josamycin propionate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Josamycin Propionate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.05 g of Josamycin Propionate in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of each peak other than josamycin propionate by the area percentage method: the amount of any peak other than josamycin is not more than 6%, and the total of these peaks is not more than 22%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 10 mL of triethylamine add water to make 1000 mL, and adjust the pH to 4.3 with acetic acid (100). To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin propionate is about 24 minutes.

Time span of measurement: About 3.5 times as long as the retention time of josamycin propionate after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 3 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of josamycin propionate obtained from 10 μL of this solution is equivalent to 8 to 12% of that from 10 μL of the solution for sys-

tem suitability test.

System performance: Dissolve 5 mg of josamycin propionate and 2 mg of josamycin in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, josamycin and josamycin propionate are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of josamycin propionate is not more than 1.5%.

Loss on drying Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Josamycin Propionate Reference Standard, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Josamycin Propionate, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

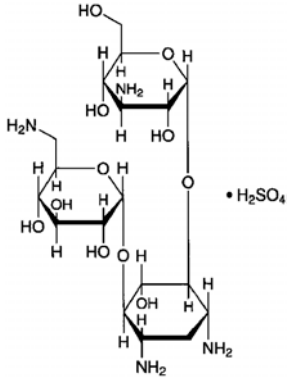
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Kanamycin Monosulfate

一硫酸カナマイシン



$C_{18}H_{36}N_4O_{11}.H_2SO_4$: 582.58

O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine monosulfate [*25389-94-0*]

Kanamycin Monosulfate contains not less than 750 μg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Monosulfate is expressed as mass (potency) of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50).

Description Kanamycin Monosulfate occurs as a white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 50 mg of Kanamycin Monosulfate in 3 mL of water, and add 6 mL of anthrone TS: a blue-purple color develops.

(2) Dissolve 20 mg each of Kanamycin Monosulfate and Kanamycin Monosulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant layer of a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R*f value.

(3) To a solution of Kanamycin Monosulfate (1 in 5) add 1 drop of barium chloride TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: +112 – +123° (0.2 g calculated on the dried basis, water, 20 mL, 100 mm).

Sulfuric acid Weigh accurately about 0.25 g of Kanamycin Monosulfate, dissolve in 100 mL of water, adjust the pH to 11.0 with ammonia solution (28), add exactly 10 mL of 0.1 mol/L barium chloride VS, and titrate with 0.1 mol/L

disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution, blue-purple, disappears (indicator: 0.5 mg of phthalein purple). At a near of the end-point add 50 mL of ethanol (99.5). Perform a blank determination in the same manner. The amount of sulfuric acid (SO_4) is not less than 15.0% and not more than 17.0%, calculated on the dried basis.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of SO_4

Purity **(1)** Heavy metals—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.30 g of Kanamycin Monosulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 45 mg of Kanamycin Monosulfate Reference Standard in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 4.0% (5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Kanamycin Monosulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Kanamycin Sulfate

硫酸カナマイシン

Change to read except the structural formula and chemical name:

Kanamycin Sulfate contains not less than 690 μg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Sulfate is expressed as mass (potency) of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50).

Description Kanamycin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** Dissolve 20 mg each of Kanamycin Sulfate and Kanamycin Monosulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R*f value.

(2) A solution of Kanamycin Sulfate (1 in 10) responds to the Qualitative Test (1) for sulfate.

Optical rotation $[\alpha]_D^{20}$: +103 – +115° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Kanamycin Sulfate in 20 mL of water is between 6.0 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Kanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Kanamycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.30 g of Kanamycin Sulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 9.0 mg of Kanamycin Monosulfate Reference Standard in water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 1 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 7.8 to 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

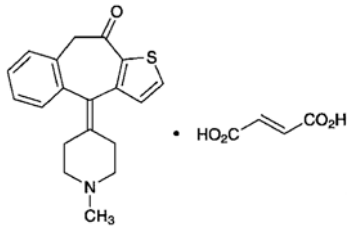
(4) Sample solutions—Weigh accurately an amount of Kanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Add the following:

Ketotifen Fumarate

フマル酸ケトチフェン



$C_{19}H_{19}NOS.C_4H_4O_4$: 425.50

4-(1-Methylpiperidin-4-ylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10(9*H*)-one monofumarate [*34580-14-8*]

Ketotifen Fumarate, when dried, contains not less than 99.0% and not more than 101.0% of ketotifen fumarate ($C_{19}H_{19}NOS.C_4H_4O_4$).

Description Ketotifen Fumarate occurs as a white to light yellowish white crystalline powder.

It is sparingly soluble in methanol and in acetic acid (100), and slightly soluble in water, in ethanol (99.5) and in acetic anhydride.

Melting point: about 190°C (with decomposition).

Identification **(1)** Prepare the test solution with 0.03 g of Ketotifen Fumarate as directed under the Oxygen Flask Combustion Method using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests for sulfate.

(2) Determine the absorption spectrum of a solution of Ketotifen Fumarate in methanol (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ketotifen Fumarate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride—Dissolve 0.6 g of Ketotifen Fumarate in 2.5 mL of sodium carbonate TS in a crucible, heat on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of sodium carbonate TS, the used amount of diluted nitric acid (3 in 10) for the neutralization, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals—Proceed with 1.0 g of Ketotifen Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ketotifen Fumarate in 10 mL of a mixture of methanol and ammonia TS (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of methanol and ammonia TS (99:1) to make exactly 25 mL. Pipet 1 mL of this solution, add a mixture of methanol and ammonia TS (99:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (90:10:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying and then hydrogen peroxide TS on the plate: the number of the spot other than the principal spot obtained from the sample solution is not more than four, and they are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.35 g of Ketotifen Fumarate, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.55 mg of $C_{19}H_{19}NOS.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Kitasamycin

キタサマイシン

Change the Content ratio of the active principle to read:

Content ratio of the active principle Dissolve 0.02 g of Kitasamycin in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 are 40 to 70%, 5 to 25% and 3 to 12%, respectively. Relative retention times of leucomycin A_4 and leucomycin A_1 to that of leucomycin A_5 are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a volume of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust to pH 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 20 mg each of Leucomycin A_5 Reference Standard and Josamycin Reference Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A_5 is not more than 1.0%.

Change the Assay (3) to read:

Assay

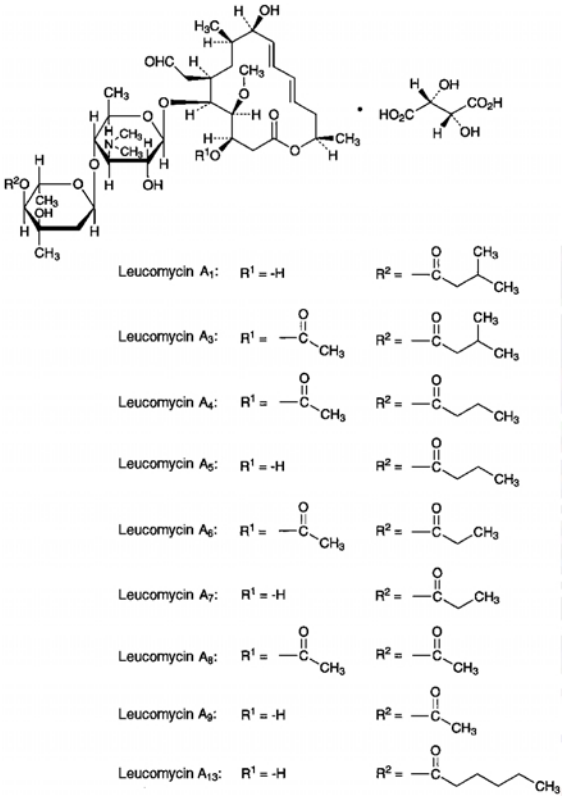
(3) Standard solutions—Weigh accurately an amount of Leucomycin A_5 Reference Standard equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

Add the following:

Kitasamycin Tartrate

Leucomycin Tartrate

酒石酸キタサマイシン



(Leucomycin A_1, A_5, A_7, A_9, A_{13} tartrate)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[*O*-(4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-(1→4)-3,6-dideoxy-3-dimethylamino-*β*-D-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate
Leucomycin A_1 tartrate: acyl = 3-methylbutanoyl
Leucomycin A_5 tartrate: acyl = butanoyl
Leucomycin A_7 tartrate: acyl = propanoyl
Leucomycin A_9 tartrate: acyl = acetyl
Leucomycin A_{13} tartrate: acyl = hexanoyl

(Leucomycin A_3, A_4, A_6, A_8 tartrate)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[*O*-(4-*O*-acyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-(1→4)-3,6-dideoxy-3-dimethylamino-*β*-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate
Leucomycin A_3 tartrate: acyl = 3-methylbutanoyl
Leucomycin A_4 tartrate: acyl = butanoyl
Leucomycin A_6 tartrate: acyl = propanoyl
Leucomycin A_8 tartrate: acyl = acetyl
[*37280-56-1*]

Kitasamycin Tartrate contains not less than 1300 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin A_5 ($C_{39}H_{65}NO_{14}$: 771.93). 1 mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin A_5 ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin Tartrate occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of *n*-butyl acetate, shake well, and discard the *n*-butyl acetate layer. To the aqueous layer add 20 mL of *n*-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Test (1) for tartrate.

pH Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

Content ratio of the active principle Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin A_5, leucomycin A_4 and leucomycin A_1 by the area percentage method: the amount of leucomycin A_5 is 40 – 70%, leucomycin A_4 is 5 – 25%, and leucomycin A_1 is 3 – 12%. The relative retention times of leucomycin A_4 and leucomycin A_1 with respect to leucomycin A_5 are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a suitable amount of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A_5 is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A_5.

System suitability—

System performance: Dissolve about 20 mg of Leucomycin A_5 Reference Standard and about 20 mg of Josamycin Reference Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A_5 and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A_5 is not more than 1.0%.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Water Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Leucomycin A_5 Reference Standard, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Latamoxef Sodium

ラタモキセフナトリウム

Change to read except the structural formula and chemical name:

Latamoxef Sodium contains not less than 830 μg (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef ($C_{20}H_{20}N_6O_9S$: 520.47).

Description Latamoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ3.5 ppm and at around δ4.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: -32 – $-40°$ (0.5 g calculated on the anhydrous basis, phosphate buffer solutiuon, pH 7.0, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1*H*-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not more than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not more than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1*H*-tetrazole-5-thiol after multiplying by its response factor, 0.52.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0%.

Water Not more than 5.0% (0.5 g, volumetric titration, back titration).

Isomer ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the areas, A_a and A_b, of the two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: A_a/A_b is between 0.8 and 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of latamoxef is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium Reference Standard, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of latamoxef to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of latamoxef } (C_{20}H_{20}N_6O_9S) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Latamoxef Ammonium Reference Standard

Internal standard solution—A solution of *m*-cresol (3 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate 12-water and 1.60 g of tetra *n*-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, latamoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

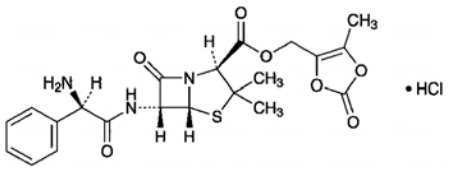
Containers and storage Containers—Tight containers.

Storage—Not exceeding 5°C.

Add the following:

Lenampicillin Hydrochloride

塩酸レナンピシリン



$C_{21}H_{23}N_3O_7S.HCl$: 497.95

5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [*80734-02-7*]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methyloxodioxolenylmethyl ester.

It contains not less than 653 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (95), and freely soluble in *N*,*N*-dimethylformamide.

Identification **(1)** Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: +174 – +194° (0.2 g calculated on the anhydrous de-residual solventization basis, ethanol (95), 20 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 2.0 g of Lenampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloridein, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak height of ampicillin to that of the internal standard: the amount of ampicillin is not more than 1.0%.

$$\text{Amount (\%) of ampicillin } (C_{16}H_{19}N_3O_4S) = \frac{W_S}{W_T} \times \frac{Q_T}{Q_S} \times 2$$

W_S: Amount [mg (potency)] of Ampicillin Reference Standard

W_T: Amount (mg) of the sample

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid ($C_{16}H_{21}N_3O_5S$: 367.42) is not more than 3.0%.

$$\text{Each mL of 0.01 mol/L sodium thiosulfate VS} = 0.45 \text{ mg of } C_{16}H_{21}N_3O_5S$$

(5) Residual solvent—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of

the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 μL each of the sample solution, the standard solution (1) and the standard solution (2) as directed under the Gas Chromatography according to the following conditions, and determine the ratios, Q_{Ta} and Q_{Tb}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, Q_{Sa1} and Q_{Sb1}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, Q_{Sa2} and Q_{Sb2}, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

$$\text{Amount (\%) of 2-propanol} = \frac{W_{Sa}}{W_T} \times \frac{2Q_{Ta} - 3Q_{Sa1} + Q_{Sa2}}{Q_{Sa2} - Q_{Sa1}}$$

$$\text{Amount (\%) of ethyl acetate} = \frac{W_{Sb}}{W_T} \times \frac{2Q_{Tb} - 3Q_{Sb1} + Q_{Sb2}}{Q_{Sb2} - Q_{Sb1}}$$

W_{Sa}: Amount (g) of 2-propanol
W_{Sb}: Amount (g) of ethyl acetate
W_T: Amount (g) of the sample

Internal standard solution—A solution of cyclohexane in *N*,*N*-dimethylformamide (1 in 1000).
Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with tetrakishydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.

Column temperature: A constant temperature of about 80°C.

Injection port temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.

System suitability—

System performance: When the procedure is run with 4 μL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5%.

Water Not more than 1.5% (1 g, volumetric titration, direct titration).

Residue on ignition Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride Reference Standard, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of lenampicillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Lenampicillin Hydrochloride Reference Standard

Internal standard solution—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lenampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Lincomycin Hydrochloride

塩酸リンコマイシン

Change to read except the structural formula and chemical name:

Lincomycin Hydrochloride contains not less than 825 μg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride is expressed as mass (potency) of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Description Lincomycin Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Lincomycin Hydrochloride as directed in the paste method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Lincomycin Hydrochloride (1 in 100) responds to the Qualitative Test (2) for chloride.

Optical rotation $[\alpha]_D^{20}$: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

pH Dissolve 0.10 g of Lincomycin Hydrochloride in 1 mL of water: 3.0 – 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lincomycin Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 2.0 g of Lincomycin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Lincomycin B—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and lincomycin B.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of lincomycin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 20 μL of the sample solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water 3.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Lincomycin Hydrochloride and Lincomycin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of lincomycin.

$$\text{Amount [}\mu\text{g (potency)] of lincomycin } (C_{18}H_{34}N_2O_6S) = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Lincomycin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 46°C.

Mobile phase: To 13.5 mL phosphoric acid add water to make 1000 mL, and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of lincomycin is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of lincomycin are not less than 4000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lincomycin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Change the Content uniformity to read:

Content uniformity Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for

15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L sodium hydroxide VS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted methanol (57 in 100).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability—

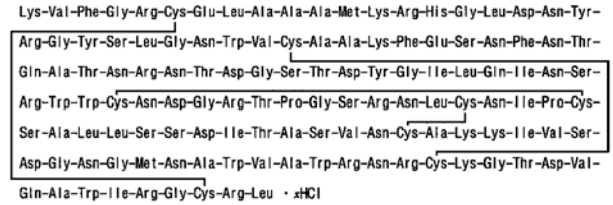
System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Add the following:

Lysozyme Hydrochloride

塩化リゾチーム



$C_{616}H_{963}N_{193}O_{182}S_{10}.x$HCl

[*12650-88-3*, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

Description Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

Identification **(1)** To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

(2) Heavy metals—Proceed with 1.0 g of Lysozyme Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying Not more than 8.0% (0.1 g, 105°C, 2 hours).

Residue on ignition Not more than 2.0% (0.5 g).

Nitrogen Perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is between 16.8% and 18.6%, calculated on the dried basis.

Assay Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution, pH 6.2 to make exactly

100 mL. Pipet 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lysozyme Reference Standard (separately determine its loss on drying in the same manner as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution, pH 6.2 to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 μL of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance, A_T, of this solution at 640 nm, using water as the blank. Determine the absorbances, A_{S1} and A_{S2}, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

$$\text{Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis} = \frac{W_S}{2W_T} \times \left(\frac{A_{S1} - A_T}{A_{S1} - A_{S2}} + 1 \right)$$

W_S: Amount (mg) of Lysozyme Reference Standard, calculated on the dried basis.

W_T: Amount (mg) of the sample, calculated on the dried basis.

Containers and storage Containers—Tight containers.

Magnesium Sulfate Injection

硫酸マグネシウム注射液

Change the Description to read:

Description Magnesium Sulfate Injection is a clear, colorless liquid.

Add the following next to Identification:

pH 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

Change the Bacterial endotoxins to read:

Bacterial endotoxins Less than 0.09 EU/mg.

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

D-Mannitol Injection

D-マンニトール注射液

Delete the Residue on ignition.

Delete the Pyrogen and add the following:

Bacterial endotoxins Less than 0.50 EU/mL.

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Menatetrenone

メナテトレノン

Change the Description to read:

Description Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about 37°C

Mepitiostane

メピチオスタン

Change the Assay to read:

Assay Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol Reference Standard, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of epitiostanol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{25}H_{40}O_2S = W_S \times \frac{Q_T}{Q_S} \times 5 \times 1.3202$$

W_S: Amount (mg) of Epitiostanol Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of *n*-octylbenzene in

ethanol (99.5) (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (20:3).

Flow rate: Adjust the flow rate so that the retention time of epitiostanol is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, epitiostanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epitiostanol to that of the internal standard is not more than 1.0%.

Methotrexate

メトトレキサート

Change the Assay to read:

Assay Weigh accurately about 25 mg each of Methotrexate and Methotrexate Reference Standard, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and measure the peak areas, A_T and A_S, of methotrexate in each solution.

$$\text{Amount (mg) of } C_{20}H_{22}N_8O_5 = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Methotrexate Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Meticrane

メチクラン

Change the Purity (4) to read:

Purity

(4) Related substances—Dissolve 0.05 g of Meticrane in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

Operating conditions 1—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (17:3).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane, after the solvent peak.

System suitability 1—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μL of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 μL of the standard solution.

System performance: Dissolve 0.01 g each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions 1, caffeine and meticrane are eluted in this order with the resolution between these peaks

being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Operating conditions 2—

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.

Time span of measurement: About 10 times as long as the retention time of meticrane, after the solvent peak.

System suitability 2—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μL of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 μL of the standard solution.

System performance: Dissolve 0.02 g each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Micronomicin Sulfate

硫酸ミクロノマイシン

Change to read except the structural formula and chemical name:

Micronomicin Sulfate contains not less than 590 μg (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micronomicin ($C_{20}H_{41}N_5O_7$: 463.57).

Description Micronomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their *R*f values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation $[\alpha]_D^{20}$: +110 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Micronomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Water Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Micronomicin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution.

Keep the standard stock solution at 5 – 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Migrenin

ミグレニン

Change the Assay (2) to read:

Assay

(2) Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine Reference Standard, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of caffeine to that of the internal standard.

$$\text{Amount (mg) of caffeine } (C_8H_{10}N_4O_2) = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Caffeine Reference Standard

Internal standard solution—A solution of ethenzamide in chloroform (1 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

System suitability—

System performance: Dissolve 0.9 g of antipyrine and 0.09 g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard is not more than 1.0%.

Minocycline Hydrochloride

塩酸ミノサイクリン

Change the Identification to read:

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Test (2) for chloride.

Delete the Absorbance.

Change the Purity to read:

Purity (1) A solution of Minocycline Hydrochloride (1 in 100) is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under the Ultraviolet-visible Spectrophotometry, is not more than 0.06.

(2) Heavy metals—Proceed with 0.5 g of Minocycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μL of the sample solution as directed under the Liquid Chro-

matography according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

Time span of measurement: About 2.5 times as long as the retention time of minocycline after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline obtained from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Mitomycin C

マイトマイシンC

Change to read except the structural formula and chemical name:

Mitomycin C contains not less than 970 μg (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C ($C_{15}H_{18}N_4O_5$).

Description Mitomycin C occurs as blue-purple, crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not more than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not more than 3 times the peak area of mitomycin C from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 10	100	0
10 – 30	100 → 0	0 → 100
30 – 45	0	100

Flow rate: About 1.0 mL/min

Time span of measurement: About 2 times as long as the retention time of mitomycin C after the solvent peak.

System suitability—

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of

methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

Loss on drying Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Mitomycin C and Mitomycin C Reference Standard, equivalent to about 25 mg (potency), dissolve each in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of mitomycin C.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{18}N_4O_5 = W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Mitomycin C Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 365 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mitomycin C is about 7 minutes.

System suitability—

System performance: Dissolve about 25 mg of Mitomycin C Reference Standard and about 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N*,*N*-dimethylacetamide. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 1.0%.

Containers and storage Containers—Tight containers.

Morphine Hydrochloride

塩酸モルヒネ

Change the Description to read:

Description Morphine Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

It is colored by light.

Add the following next to Optical rotation:

pH The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride in 10 mL of water is between 4.0 and 6.0.

Change the Purity to read:

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Morphine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Dissolve 0.20 g of Morphine Hydrochloride in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Other alkaloids—Dissolve 0.1 g of Morphine Hydrochloride in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Morphine Hydrochloride Injection

塩酸モルヒネ注射液

Change the Assay to read:

Assay Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$), and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution.

Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine hydrochloride } (C_{17}H_{19}NO_3.HCl.3H_2O) = W_S \times \frac{Q_T}{Q_S} \times 4 \times 1.1679$$

W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Morphine Hydrochloride Tablets

塩酸モルヒネ錠

Change the Assay to read:

Assay Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine hydrochloride } (C_{17}H_{19}NO_3.HCl.3H_2O) = W_S \times \frac{Q_T}{Q_S} \times 1.1679$$

W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Norepinephrine

ノルエピネフリン

Change the Description to read:

Description Norepinephrine occurs as a white to light brown or slightly reddish brown, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

It gradually changes to brown by air and by light.

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Norepinephrine in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Norepinephrine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Norepinephrine Injection

ノルエピネフリン注射液

Change the Identification to read:

Identification Transfer a volume of Norepinephrine Injection, equivalent to 1 mg of Norepinephrine according to the labeled amount, to each of two test tubes A and B, and add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and 10 mL of phosphate buffer solution, pH 6.5, to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

Norgestrel and Ethinylestradiol Tablets

ノルゲストレル・エチニルエストラジオール錠

Change the Content uniformity to read:

Content uniformity Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel Reference Standard and of Ethinylestradiol Reference Standard, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

Amount (mg) of norgestrel ($C_{21}H_{28}O_2$)

$$= W_{Sa} \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{100}$$

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)

$$= W_{Sb} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{100}$$

W_{Sa}: Amount (mg) of Norgestrel Reference Standard
W_{Sb}: Amount (mg) of Ethinylestradiol Reference Standard

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Change the Dissolution test to read:

Dissolution test Perform the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the test solution. Take 50 mL or more of the dissolved solution 45 minutes after starting the test, and membrane filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, transfer exactly 30 mL of the subsequent into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent on a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel Reference Standard and about 2.5 mg of Ethinylestradiol Reference Standard dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_{Ta} and A_{Tb}, of norgestrel and ethinylestradiol from the sample solution, and the peak areas, A_{Sa} and A_{Sb}, of norgestrel and ethinylestradiol from the standard solution.

The dissolution rate of Norgestrel and Ethinylestradiol Tablets in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of norgestrel ($C_{21}H_{28}O_2$)

$$= W_{Sa} \times \frac{A_{Ta}}{A_{Sa}} \times \frac{1}{C_a} \times \frac{9}{5}$$

Dissolution rate (%) with respect to the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$)

$$= W_{Sb} \times \frac{A_{Tb}}{A_{Sb}} \times \frac{1}{C_b} \times \frac{9}{5}$$

W_{Sa}: Amount (mg) of Norgestrel Reference Standard

W_{Sb}: Amount (mg) of Ethinylestradiol Reference Standard

C_a: Labeled amount (mg) of norgestrel ($C_{21}H_{28}O_2$) in 1 tablet

C_b: Labeled amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Change the Assay to read:

Assay Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel ($C_{21}H_{28}O_2$), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel Reference Standard and about 5 mg of Ethinylestradiol Reference Standard, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

Amount (mg) of norgestrel ($C_{21}H_{28}O_2$)

$$= W_{Sa} \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{50}$$

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)

$$= W_{Sb} \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{50}$$

W_{Sa}: Amount (mg) of Norgestrel Reference Standard

W_{Sb}: Amount (mg) of Ethinylestradiol Reference Standard

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Detector: Norgestrel—An ultraviolet absorption photometer (wavelength: 241 nm).

Ethinylestradiol—A fluorophotometer (excitation wavelength: 281 nm, fluorescence wavelength: 305 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of norgestrel is about 10 minutes.

System suitability—

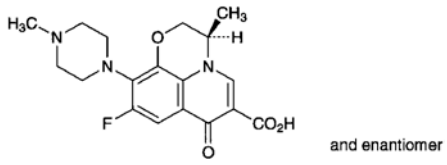
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

Add the following:

Ofloxacin

オフロキサシン



and enantiomer

$C_{18}H_{20}FN_3O_4$: 361.37

(3*RS*)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid [*82419-36-1*]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin ($C_{18}H_{20}FN_3O_4$).

Description Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A soluton of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 265°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of

a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ofloxacin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals—Proceed with 2.0 g of Ofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin obtained from the sample solution is not more than 0.4 times the peak area of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin from the sample solution is not more than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 294 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ofloxacin is about 20 minutes.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin after the solvent peak.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that from 10 μL of the standard solution.

System performance: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

Loss on drying Not less than 0.2% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.30 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxytetracycline Hydrochloride

塩酸オキシテトラサイクリン

Change to read except the structural formula and chemical name:

Oxytetracycline Hydrochloride contains not less than 880 μg (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.43).

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Oxytetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation $[\alpha]_D^{20}$: -188 – $-200°$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of β-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as β-apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of β-apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline obtained from the sample solution are not more than each of the peak area obtained from the standard solution, and the total area of the peaks, α-apooxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, β-apooxytetracycline and the peaks, which appear between α-apooxytetracycline and β-apooxytetracycline, is not more than the peak area of β-apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak, obtained from the sample solution is not more than 4 times the peak area of 4-epioxytetracycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of *t*-butanol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of *t*-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 20	70 → 10	30 → 90
20 – 35	10 → 20	90 → 80

Flow rate: 1.0 mL/min

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α-apooxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epioxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β-apooxytetracycline stock solution and 6 mL of α-apooxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-epioxytetracycline, oxytetracycline, tetracycline, α-apooxytetracycline and β-apooxytetracycline are eluted in this order with the resolutions between the peaks, 4-epioxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and α-apooxytetracycline and β-apooxytetracycline being not less than 4, not less than 5 and not less than 4, respectively, and the symmetry coefficient of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epioxytetracycline is not more than 2.0%.

Loss on drying Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these

solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of oxytetracycline.

Amount [μg (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$)

$$= W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Oxytetracycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Peplomycin Sulfate

硫酸ペプロマイシン

Change to read except the structural formula and chemical name:

Peplomycin Sulfate contains not less than 865 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Description Peplomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate Reference Standard in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions: the retention time of the principal spot obtained from the sample solution is the same as that from the standard soution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds to the Qualitative Tests (1) and (2) for sulfate.

Optical rotation $[\alpha]_D^{20}$: -2 – $-5°$ (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene
Supporting gas—Air

Lamp: Copper hollow cathode lamp

Wavelength: 324.8 nm

(3) Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine the areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calcu-

late the amounts of them by the area percentage method: the total amount of the peaks other than peplomycin is not more than 7%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin after the peak of copper sulfate.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

Loss on drying Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Mycobacterium smegmatis* ATCC 607

(2) Agar media for seed and base layer, and for transferring test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(3) Liquid medium for suspending test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(4) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours. Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, incubate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(5) Preparation of cylinder-agar plate—Proceed as directed in 7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

(6) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(7) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (poten-

cy) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers

Pethidine Hydrochloride

塩酸ペチジン

Change the Purity (3) to read:

Purity

(3) Related substances—Dissolve 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than that of pethidine from the sample solution is not larger than the peak area of perthidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 μL of this solution is equivalent to 5 to 15% of that of pethidine obtained from 20 μL of the standard solution.

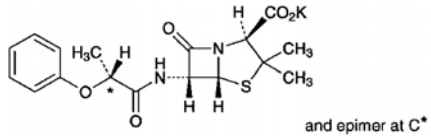
System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

Add the following:

Phenethicillin Potassium

フェネチシリンカリウム



$C_{17}H_{19}KN_2O_5S$: 402.51

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*RS*)-2-phenoxypropanoylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*132-93-4*]

Phenethicillin Potassium contains not less than 1400 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$). 1 unit of Phenethicillin Potassium is equivalent to 0.68 μg of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$).

Description Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Phenethicillin Potassium (1 in 5000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Phenethicillin Potassium responds to the Qualitative Test (1) for potassium salt.

Optical rotation $[\alpha]_D^{20}$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

L-α-Phenethicillin potassium Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_D and A_L, of D-α-phenethicillin and L-α-phenethicillin by the automatic integration method: $A_L/(A_D + A_L)$ is between 0.50 and 0.70.

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Adjust the pH of a mixture of a solution of diammonium hydrogen phosphate (1 in 150) and acetonitrile (41:10) to 7.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of L-α-phenethicillin is about 25 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, D-α-phenethicillin and L-α-phenethicillin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of L-α-phenethicillin is not more than 2.0%.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Phenethicillin Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Phenethicillin Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than D-α-phenethicillin and L-α-phenethicillin obtained from the sample solution is not more than 5 times the total of the peak areas of D-α-phenethicillin and L-α-phenethicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the L-α-Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-α-phenethicillin obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the L-α-Phenethicillin potassium.

Loss on drying Not more than 1.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Phenethicillin Potassium and dried L-Phenethicillin Potassium Reference Standard, equivalent to about 40,000 units, dissolve each in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 2 mL each of these solutions in 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS to them, and allow to stand for exactly 15 minutes. To them add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, and allow them to stand for exactly 15 minutes. Add 0.2 – 0.5 mL of starch TS, and titrate with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and the standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above without allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, V_T and V_S, of 0.005 mol/L iodine VS consumed in the sample solution and the standard solution.

$$\text{Amount (unit) of } C_{17}H_{19}KN_2O_5S = W_S \times \frac{V_T}{V_S}$$

W_S: Amount (unit) of L-Phenethicillin Potassium Reference Standard

Containers and storage Containers—Well-closed containers.

Phytonadione

フィトナジオン

Change the Description to read:

Description Phytonadione is a clear yellow to orange-yellow, viscous liquid.

It is miscible with isooctane.

It is sparingly soluble in ethanol (99.5), and practically insoluble in water.

It decomposes gradually and changes to a red-brown by light.

Specific gravity d_{20}^{20}: about 0.967

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 10,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phytonadione as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Change the Purity to read:

Purity **(1)** Ratio of absorbances—Determine the absorbances, A_1, A_2 and A_3, of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively: the ratio A_2/A_1 is between 0.69 and 0.73, and the ratio A_2/A_3 is between 0.74 and 0.78. Determine the absorbances, A_4 and A_5, of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio A_4/A_5 is between 0.28 and 0.34.

(2) Heavy metals—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

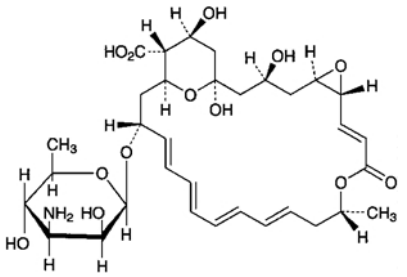
(3) Menadione—Dissolve 0.020 g of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

Add the following:

Pimaricin

Natamycin

ピマリシン



$C_{33}H_{47}NO_{13}$: 665.73

(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [*7681-93-8*]

Pimaricin contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin ($C_{33}H_{47}NO_{13}$).

Description Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

Identification **(1)** To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation $[\alpha]_D^{20}$: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 303 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

Flow rate: Adjust the flow rate so that the retention time of pimaricin is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pimaricin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of pimaricin is not more than 2.0%.

Water Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pimaricin and Pimaricin Reference Standard, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL each of these solution, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Determine the absorbances at 295.5 nm, A_{T1} and A_{S1}, at 303 nm, A_{T2} and A_{S2}, and at 311 nm, A_{T3} and A_{S3}, of the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount [}\mu\text{g (potency)] of } C_{33}H_{47}NO_{13} = W_S \times \frac{A_{T2} - \dfrac{A_{T1} + A_{T3}}{2}}{A_{S2} - \dfrac{A_{S1} + A_{S3}}{2}} \times 1000$$

W_S: Amount [mg (potency)] of Pimaricin Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light resistant.

Pipemidic Acid Trihydrate

ピペミド酸三水和物

Change the origin/limits of content to read:

Pipemidic Acid Trihydrate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{14}H_{17}N_5O_3$ (mol. wt.: 303.32).

Change the Description to read:

Description Pipemidic Acid Trihydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Melting point: about 250°C (with decomposition).

Change the Identification to read:

Identification **(1)** Dissolve 0.1 g of Pipemidic Acid Trihydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pipemidic Acid Trihydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Change the Assay to read:

Assay Weigh accurately about 0.3 g of Pipemidic Acid Trihydrate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Piperacillin Sodium

ピペラシリンナトリウム

Change the Description to read:

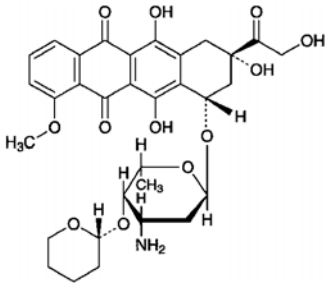
Description Piperacillin Sodium occurs as a white powder or mass.

It is very soluble in water, freely soluble in methanol and in ethanol (95), and practically insoluble in acetonitrile.

Add the following:

Pirarubicin

ピラルビシン



$C_{32}H_{37}NO_{12}$: 627.64

(2*S*,4*S*)-4-{3-Amino-2,3,6-trideoxy-4-*O*-[(2*R*)-tetrahydropyran-2-yl]-α-L-*lyxo*-hexopyranosyloxy}-1,2,3,4-tetrahydro-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxynaphthacene-6,11-dione [*72496-41-4*]

Pirarubicin contains not less than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin ($C_{32}H_{37}NO_{12}$).

Description Pirarubicin occurs as a red-orange crystalline powder.

It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Pirarubicin and Pirarubicin Reference Standard in 5 mL of chloroform, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same *R*f value.

Optical rotation $[\alpha]_D^{20}$: +195 – +215° (10 mg, chloroform, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

(2) Heavy metals—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not more than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not more than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their response factors, 1.09, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water Not more than 2.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pirarubicin and Pirarubicin Reference Standard, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of pirarubicin to that of the internal standard.

Amount [μg (potency)] of $C_{32}H_{37}NO_{12}$ = $W_S \times \frac{Q_T}{Q_S} \times 1000$

W_S: Amount [mg (potency)] of Pirarubicin Reference Standard

Internal standard solution—A solution of 2-naphthol in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L ammonium formate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Pivmecillinam Hydrochloride

塩酸ピブメシリナム

Change to read except the structural formula and chemical name:

Pivmecillinam Hydrochloride contains not less than 630 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of pivmecillinam ($C_{21}H_{33}N_3O_5S$: 439.57).

Description Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity (1) Heavy metals—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride Reference Standard in 4.0 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

Water Not more than 1.0% (0.25 g, coulometric titration).

Assay Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of pivmecillinam to that of the internal standard.

Amount [μg (potency)] of pivmecillinam ($C_{21}H_{33}N_3O_5S$)
$$= W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Pivmecillinam Hydrochloride Reference Standard

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Polymixin B Sulfate

硫酸ポリミキシン B

Change to read except the structural formula and chemical name:

Polymixin B Sulfate contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B Sulfate is expressed as mass unit of polymixin B ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}$). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}.1\text{-}2H_2SO_4$).

Description Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate Reference Standard separately in two glass stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and the standard solution (1). Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine separately in 10 mL of water, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 3 μL each of the sample solution, the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5) on a plate of silica gel for thin-layer chromatography, and expose the plate to a saturated vapor of the developing solvent for 15 hours. Develop the plate with a mixture of phenol and water (3:1) to a distance of about 13 cm while without exposure to light, and dry the plate at 110°C for 5 minutes. Spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110°C for 5 minutes: *R*f value of each spot obtained from the sample solution is the same with *R*f value of the corresponding spots from the standard solution (1). Each of the spots from the sample solution appears at the position corresponding to each of the spots from the standard (2), (3) and (4), but not appears at the position corresponding to the spot from the standard solution (5).

(3) A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests for sulfate.

Optical rotation $[\alpha]_D^{20}$: -78 – $-90°$ (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

Phenylalanine Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine absorbances, A_1, A_2, A_3, A_4 and A_5, of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under the Ultraviolet-visible Spectrophotometry, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine calculated on the dried basis is not less than 9% and not more than 12%.

Amount (%) of phenylalanine
$$= \frac{A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5}{W_T} \times 9.4787$$

W_T: Amount (g) of the sample, calculated on the dried basis

Purity Heavy metals—Proceed with 1.0 g of Polymixin B Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead

Solution (not more than 20 ppm).

Loss on drying Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 0.75% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Escherichia coli* NIHJ

(2) Agar media for seed and base layer

Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate Reference Standard, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Potassium Clavulanate

クラブラン酸カリウム

Change the origin/limits of content to read:

Potassium Clavulanate contains not less than 810 μg (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavularic acid ($C_8H_9NO_5$: 199.16).

Change the Identification (1) to read:

Identification **(1)** To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Change the Optical rotation to read:

Optical rotation $[\alpha]_D^{20}$: +53 – +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Delete the pH.

Add the following next to Purity (2):

Purity

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not more than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not more than 2 times of the peak area of clavulanic acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A and methanol (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 4	100	0
4 – 15	100 → 0	0 → 100
15 – 25	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10

mL. Confirm that the peak area of clavulanic acid obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: Dissolve 10 mg each of Potassium Clavulanate and Amoxycillin Reference Standard in 100 mL of the mobile phase A. When the procedure is run with 20 μL of this solution under the above operating conditions, clavulanic acid and amoxycillin are eluted in this order with the resolution between these peaks being not less than 8 and the number of theoretical plates of the peak of clavulanic acid is not less than 2500 steps.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

Povidone-Iodine

ポビドンヨード

Change the Description to read:

Description Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

Change the Purity (3) to read:

Purity

(3) Arsenic—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

Change the Assay (2) to read:

Assay

(2) Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under the Nitrogen Determination.

Prednisolone Acetate

酢酸プレドニゾロン

Change the Assay to read:

Assay Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate Reference Standard, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the text with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of prednisolone acetate to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{30}O_6 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Prednisolone Acetate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of prednisolone acetate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

Prednisolone Sodium Succinate for Injection

注射用コハク酸プレドニゾロンナトリウム

Change the Assay to read:

Assay Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.10 g of prednisolone ($C_{21}H_{28}O_5$), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate Reference Standard, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution.

Perform the test with 10 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prednisolone succinate to that of the internal standard.

Amount (mg) of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$)

$$= W_S \times \frac{Q_T}{Q_S} \times 5 \times 1.0477$$

Amount (mg) of prednisolone ($C_{21}H_{28}O_5$)

$$= W_S \times \frac{Q_T}{Q_S} \times 5 \times 0.7827$$

W_S: Amount (mg) of Prednisolone Succinate Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of disodium hydrogen phosphate 12-water and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.
System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

Primidone

プリミドン

Change the Purity (3) to read:

Purity

(3) 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μL of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: Q_T is not more than Q_S.
Internal standard solution—A solution of stearylalcohol in pyridine (1 in 2000).
Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of stearylalcohol is about 10 minutes.
System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating condition, 2-ethyl-2-phenylmalonediamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

Procaine Hydrochloride Injection

塩酸プロカイン注射液

Change the Assay to read:

Assay To an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under

the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of procaine to that of the internal standard.

Amount (mg) of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl)

$$= W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of procaine hydrochloride for assay

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of procaine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of procaine to that of the internal standard is not more than 1.0%.

Procarbazine Hydrochloride

塩酸プロカルバジン

Change the origin/limits of content to read:

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{12}H_{19}N_3O$.HCl.

Change the Description to read:

Description Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

Change the Purity to read:

Purity **(1)** Heavy metals—Proceed with 1.0 g of Procarbazine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Immerse slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μL each of the sample solution and the standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is not more intense than the spot from the standard solution.

Pyrazinamide

ピラジナミド

Change the origin/limits of content to read:

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of $C_5H_5N_3O$.

Change the Description to read:

Description Pyrazinamide occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Pyrazinamide in 100 mL of water is between 5.0 and 7.0.

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium

bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Change the Purity (4) and (5) to read:

Purity

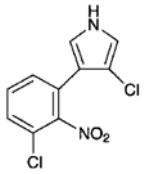
(4) Related substances—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 20 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(5) Readily carbonizable substances—Perform the test with 0.20 g of Pyrazinamide: the solution has no more color than Matching Fluid A.

Add the following:

Pyrrolnitrin

ピロールニトリン



$C_{10}H_6Cl_2N_2O_2$: 257.07

3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole [*1018-71-9*]

Pyrrolnitrin contains not less than 970 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin ($C_{10}H_6Cl_2N_2O_2$).

Description Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 124 – 128°C

Purity Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin Reference Standard, equivalent to about 50 mg (potency), dissolve in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of pyrrolnitrin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{10}H_6Cl_2N_2O_2 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Pyrrolnitrin Reference Standard

Internal standard solution—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, pyrrolnitrin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

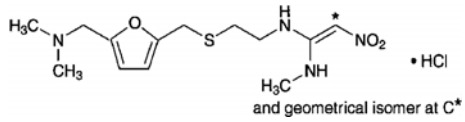
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Ranitidine Hydrochloride

塩酸ラニチジン



$C_{13}H_{22}N_4O_3S.HCl$: 350.86

N-{2-[({5-[(Dimethylamino)methyl]furan-2-yl}methyl)sulfanyl]ethyl}-*N'*-methyl-2-nitroethene-1,1-diamine monohydrochloride [*66357-59-3*]

Ranitidine Hydrochloride, when dried, contains not less than 97.5% and not more than 102.0% of ranitidine hydrochloride ($C_{13}H_{22}N_4O_3S.HCl$).

Description Ranitidine Hydrochloride occurs as a white to pale yellow, crystalline or fine granular powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

It is gradually colored by light.

Melting point: about 140°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ranitidine Hydrochloride (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ranitidine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ranitidine Hydrochloride as directed in the paste method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Ranitidine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH The pH of a solution obtained by dissolving 1.0 g of Ranitidine Hydrochloride in 100 mL of water is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—A solution of Ranitidine Hydrochloride (1 in 10) is clear and pale yellow to light yellow.

(2) Heavy metals—Proceed with 2.0 g of Ranitidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Ranitidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.22 g of Ranitidine Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 6 mL, 4 mL, 2 mL and 1 mL of the standard solution (1), add to each methanol to make exactly 10 mL, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Separately, dissolve 12.7 mg of ranitidinediamine in methanol to make exactly 10 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography. Separately, spot 10 μL of the sample solution on the plate, then spot 10 μL of the standard solution (6) on the spotted position of the sample solution. Immediately develop the plate with a mixture of ethyl acetate, 2-propanol, ammonia solution (28) and water (25:15:5:1) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand in iodine vapor until the spot from the standard solution (5) appears: the spot obtained from the standard solution (6) is completely separated from the principal spot from the sample solution. The spot having *R*f value of about 0.7 from the sample solution is not more intense than the spot from the standard solution (1), the spots other than the principal spot and the spot of *R*f 0.7 from the sample solution are not more intense than the spot from the standard solution (2), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 1.0%.

Loss on drying Not more than 0.75% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 20 mg of Ranitidine Hydrochloride and Ranitidine Hydrochloride Reference

Standard, previously dried, dissolve each in the mobile phase to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of ranitidine.

$$\text{Amount (mg) of } C_{13}H_{22}N_4O_3S.HCl = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Ranitidine Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 322 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 5) (17:3).

Flow rate: Adjust the flow rate so that the retention time of ranitidine is about 5 minutes.

System suitability—

System performance: Dissolve 20 mg of Ranitidine Hydrochloride and 5 mg of benzalphthalide in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, benzalphthalide and ranitidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ranitidine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Reserpine

レセルピン

Change the Purity to read:

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total area of all peaks other than reserpine peak from the sample solution is not larger than the peak area of reserpine from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 20 minutes.

Time span of measurement: About twice as long as the retention time of reserpine.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add acetonitorile to make exactly 50 mL. Confirm that the peak area of reserpine obtained from 10 μL of this solution is equivalent to 3 to 5% of that of reserpine obtained from 10 μL of the standard solution.

System performance: Dissolve 0.01 g of Reserpine and 4 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL. When the procedure is run with 20 μL of this solution according to the operating conditions in the Assay, reserpine and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of reserpine is not more than 2.0%.

Change the Assay to read:

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 10 mg each of Reserpine and Reserpine Reference Standard, previously dried, and dissolve each in acetonitrile to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of reserpine to that of the internal standard.

$$\text{Amount (mg) of } C_{33}H_{40}N_2O_9 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Reserpine Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, reserpine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of reserpine to that of the internal standard is not more than 2.0%.

Retinol Acetate

酢酸レチノール

Change to read except the structural formula and chemical name:

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil. It contains not less than 2,500,000 Vitamin A Units per gram. A suitable antioxidant may be added.

Retinol Acetate contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

It is freely soluble in petroleum ether, soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Acetate and Retinol Acetate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and *R*f value with the blue spot from the standard solution.

Purity **(1)** Acid value—Take exactly 5.0 g of Retinol Acetate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Acetate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

$$\text{Amount (meq/kg) of peroxide} = \frac{V}{W} \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed

W: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Retinol Palmitate

パルミチン酸レチノール

Change to read except the structural formula and chemical name:

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil, and contains not less than 1,500,000 Vitamin A Units in each gram. It may contain a suitable antioxidant.

Retinol Palmitate contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

It is very soluble in petroleum ether, slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Palmitate and Retinol Palmitate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and *R*f value with the blue spot from the standard solution.

Purity **(1)** Acid value—Take exactly 5.0 g of Retinol

Palmitate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Palmitate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

$$\text{Amount (meq/kg) of peroxide} = \frac{V}{W} \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS
W: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Ribostamycin Sulfate

硫酸リボスタマイシン

Change to read except the structural formula and chemical name:

Ribostamycin Sulfate contains not less than 680 μg (potency) per mg, calculated on the dried basis. The potency of Ribostamycin Sulfate is expressed as mass (potency) of ribostamycin ($C_{17}H_{34}N_4O_{10}$: 454.47).

Description Ribostamycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Dissolve 20 mg of Ribostamycin Sulfate in 2 mL of phosphate buffer solution, pH 6.0, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 0.12 g each of Ribostamycin Sulfate and Ribostamycin Sulfate Reference Standard in 20 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same Rf value.

(3) To 2 mL of a solution of Ribostamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation $[\alpha]_D^{20}$: +42 – +49° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 1.0 g of Ribostamycin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ribostamycin Sulfate in 5 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.12 g of Ribostamycin Sulfate in water to make exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Ribostamycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 20 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains

20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rifampicin

リファンピシン

Change to read except the structural formula and chemical name:

Rifampicin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Rifampicin is expressed as mass (potency) of rifampicin ($C_{43}H_{58}N_4O_{12}$: 822.94).

Description Rifampicin occurs as orange-red to red-brown, crystals or crystalline powder.

It is slightly soluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification **(1)** To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rifampicin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rifampicin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Rifampicin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Rifampicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Rifampicin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Perform the test immediately after preparing of the sample and standard solutions. Dissolve 0.10 g of Rifampicin in 50 mL of acetonitrile, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 1 mL of the sample stock solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak appeared at the relative retention time of about 0.7 with respect to rifampicin from the sample solution is not more than 1.5 times the peak area of rifampicin from the standard solution, the area of the peak other than rifampicin and the peak mentioned above from the sample solution is not more than the peak area of rifampicin from the standard solution, and the total area of the peaks other than rifampicin and the peak mentioned above from the sample solution is not more than 3.5 times the peak area of rifampicin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of rifampicin after the peak of the solvent.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add citric acid-phosphate-acetonitrile TS to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 50 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0%.

Loss on drying Not more than 2.0% (1 g, reduced pressure not exceeding 0.69 kPa, 60°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately an amount of Rifampicin and Rifampicin Reference Standard, equivalent to about 40 mg (potency), and dissolve each in acetonitrile to make exactly 200 mL. Pipet 10 mL each of these solutions, add citric acid-phosphate-acetonitrile TS to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 50 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of rifampicin.

Amount [μg (potency)] of $C_{43}H_{58}N_4O_{12}$

$$= W_S \times \frac{A_T}{A_S} \times 1000$$

W_S: Amount [mg (potency)] of Rifampicin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in 1000 mL of a mixture of water, acetonitrile and phosphate buffer solution, pH 3.1 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 8 minutes.

System suitability—

System performance: To 5 mL of a solution of Rifampicin in acetonitrile (1 in 5000) add 1 mL of a solution of butyl parahydroxybenzoate in acetonitrile (1 in 5000) and citric acid-phosphate-acetonitrile TS to make 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Roxithromycin

ロキシスロマイシン

Change the Description to read:

Description Roxithromycin occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, sparingly soluble in acetonitrile, and practically insoluble in water.

Scopolamine Butylbromide

臭化ブチルスコポラミン

Change the Purity (3) to read:

Purity

(3) Related substances—Dissolve 0.10 g of Scopolamine Butylbromide in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of scopolamine hydrobromide in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of scopolamine from the sample solution is not larger than that from the standard solution (2), and each area of the peaks other than the peak appearing in the first elution and the peak of scopolamine and butylscopolamine from the sample solution are not larger than the peak area from the standard solution (1).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in 370 mL of water and 680 mL of methanol, and adjust the pH to 3.6 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust the flow rate so that the retention time of butylscopolamine is about 7 minutes.

Time span of measurement: About twice as long as the retention time of butylscopolamine.

System suitability—

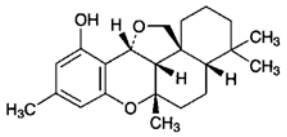
System performance: Dissolve 5 mg each of Scopolamine Butylbromide and scopolamine hydrobromide in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, scopolamine and butylscopolamine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of scopolamine is not more than 2.0%.

Add the following:

Siccanin

シッカニン



$C_{22}H_{30}O_3$: 342.47

(4a*S*,6a*S*,11b*R*,13a*S*,13b*S*)-1,2,3,4,4a,5,6,6a,11b,13b-Decahydro-4,4,6a,9-tetramethyl-13*H*-benzo[*a*]furo[2,3,4-*mn*]xanthen-11-ol [*22733-60-4*]

Siccanin contains not less than 980 μg (potency) per mg, calculated on the dried basis. The potency of Siccanin is expressed as mass (potency) of siccanin ($C_{22}H_{30}O_3$).

Description Siccanin occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Siccanin in ethanol (99.5) (1 in 10,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Siccanin Reference Standard obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Siccanin as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Siccanin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: -165 – $-175°$ (0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point 138 – 142°C

Purity **(1)** Heavy metals—Proceed with 1.0 g of Siccanin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Siccanin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-chlorobenzenediazonium TS on the plate: the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 80°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately an amount of Siccanin and Siccanin Reference Standard, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of siccanin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{22}H_{30}O_3 = W_S \times \frac{Q_T}{Q_S} \times 1000$$

W_S: Amount [mg (potency)] of Siccanin Reference Standard

Internal standard solution—A solution of 1,4-diphenylbenzene in methanol (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and phosphate buffer solution, pH 5.9 (19:6).

Flow rate: Adjust the flow rate so that the retention time of siccanin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, siccanin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of siccanin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Delete the following Monograph:

Simfibrate

シンフィブラート

Sisomicin Sulfate

硫酸シソマイシン

Change the Purity (3) to read:

Purity

(3) Related substances—Dissolve 50 mg of Sisomicin Sulfate, calculated on the dried basis, in water to make 10 mL, and use this solution as the sample solution. Pipet 0.5 mL, 1 mL and 1.5 mL of the sample solution, add water to each to make exactly 50 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions (1), (2), and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butabol TS on the plate, and heat at 100°C for 5 minutes. The spots corresponding to *R*f about 0.35 and *R*f about 0.30 are not more intense than that of the spot from the standard solution (3), and the spot of gallamine corresponding to *R*f about 0.25 is not more intense than the spot from the standard solution (1). The total amount of the related substances is not more than 6%.

Sodium Bicarbonate Injection

炭酸水素ナトリウム注射液

Add the following next to Identification:

pH 7.0 – 8.5

Delete the Purity.

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Change to read:

Sodium Chloride

塩化ナトリウム

NaCl: 58.44

Sodium Chloride, when dried, contains not less than 99.0% and not more than 100.5% of NaCl.

Description Sodium Chloride occurs as colorless or white, crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sodium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 20.0 g of Sodium Chloride in 100.0 mL of freshly boiled and cooled water, and use this solution as the sample solution. To 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow. Separately, to 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is blue.

(3) Sulfates—To 7.5 mL of the sample solution obtained in (2) add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minutes. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: any turbidity produced does not more than that produced in the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as directed above using this solution instead of the sample solution.

(4) Phosphates—To 2.0 mL of the sample solution obtained in (2) add 5 mL of 2 mol/L sulfuric acid TS and water to make 100.0 mL, then add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes: the color of the solution is not darker than the following control solution.

Control solution: To 1.0 mL of Standard Phosphoric Acid Solution add 12.5 mL of 2 mol/L sulfuric acid TS and

water to make exactly 250 mL. To 100 mL of this solution add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes.

(5) Bromides—To 0.50 mL of the sample solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. After allowing to stand for 2 minutes, add 0.15 mL of 0.1 mol/L sodium thiosulfate VS, mix, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1,000,000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Ultraviolet-visible Spectrophotometry using water as the control: the absorbance at 590 nm of the sample solution is not more than that of the standard solution.

(6) Iodides—Wet 5 g of Sodium Chloride with dropwisely added 0.15 mL of a freshly prepared mixture of starch TS, 0.5 mol/L sulfuric acid TS and sodium nitrite TS (1000:40:3), allow to stand for 5 minutes, and examine under daylight: a blue color does not appear.

(7) Ferrocyanides—Dissolve 2.0 g of Sodium Chloride in 6 mL of water, and add 0.5 mL of a mixture of a solution of iron (II) sulfate heptahydrate (1 in 100) and a solution of ammonium iron (III) sulfate 12-water in diluted sulfuric acid (1 in 400) (1 in 100) (19:1): a blue color does not develop within 10 minutes.

(8) Heavy metals—Proceed with 5.0 g of Sodium Chloride according to Method 1, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 3 ppm).

(9) Iron—To 10 mL of the sample solution obtained in (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, alkalize with ammonia TS, add water to make 20 mL, and allow to stand for 5 minutes: the solution has not more color than the following control solution.

Control solution: Pipet 1 mL of Standard Iron Solution, and add water to make exactly 25 mL. To 10 mL of this solution add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, and proceed in the same manner as directed for the sample solution.

(10) Barium—To 5.0 mL of the sample solution obtained in (2) add 5.0 mL of water and 2.0 mL of dilute sulfuric acid, and allow to stand for 2 hours: the solution has not more turbidity than the following control solution.

Control solution: To 5.0 mL of the sample solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.

(11) Magnesium and alkaline-earth materials—To 200 mL of water add 0.1 g of hydroxylammonium chloride, 10 mL of ammonium chloride buffer solution, pH 10, 1 mL of 0.1 mol/L zinc sulfate VS and 0.2 g of eriochrome black T-sodium chloride indicator, and warm to 40°C. Add 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS dropwise until the red-purple color of the solution changes to blue-purple. To this solution add a solution prepared by dissolving 10.0 g of Sodium Chloride in 100 mL of water, and add 2.5 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

(12) Arsenic—Prepare the test solution with 1.0 g of Sodium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, previously dried, dissolve in 50 mL of water, and titrate with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and storage Containers—Tight containers.

10% Sodium Chloride Injection

10% 塩化ナトリウム注射液

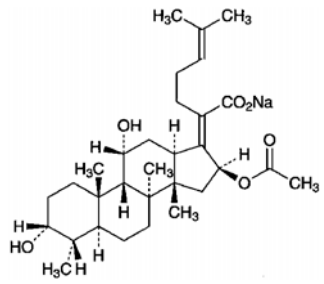
Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Add the following:

Sodium Fusidate

フシジン酸ナトリウム



$C_{31}H_{47}NaO_6$: 538.69
Monosodium (17Z)-*ent*-16α-acetoxy-3β,11β-dihydroxy-4β,8β,14α-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oate [*751-94-0*]

Sodium Fusidate contains not less than 935 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sodium Fusidate is expressed as mass (potency) of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Description Sodium Fusidate occurs as white, crystals of

crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectra of Sodium Fusidate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sodium Fusidate responds to the Qualitative Test (1) for sodium salt.

Purity Heavy metals—Proceed with 2.0 g of Sodium Fusidate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Water Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Staphylococcus aureus* ATCC 6538 P

(2) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Diethanolamine Fusidate Reference Standard, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Sodium Fusidate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature 2 to 8°C.

Delete the following Monographs:

Sodium Iopodate

イオポダートナトリウム

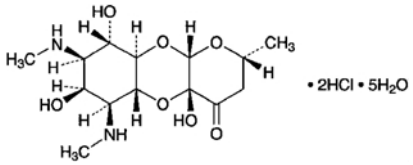
Sodium Iopodate Capsules

イオポダートナトリウムカプセル

Add the following:

Spectinomycin Hydrochloride

塩酸スペクチノマイシン



$C_{14}H_{24}N_2O_7.2HCl.5H_2O$: 495.35

(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 [*22189-32-8*]

Spectinomycin Hydrochloride contains not less than 603 μg (potency) per mg. The potency of Spectinomycin Hydrochloride is expressed as mass (potency) of spectinomycin ($C_{14}H_{24}N_2O_7$: 332.35).

Description Spectinomycin Hydrochloride occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** To 5 mL of a solution of Spectinomycin Hydrochloride (1 in 100) add gently anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared absorption spectra of Spectinomycin Hydrochloride and Spectinomycin Hydrochloride Reference Standard as directed in the paste method under the Infrared Spectrophotometry, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 mL of a solution of Spectinomycin Hydrochloride (1 in 150) add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation $[\alpha]_D^{20}$: +15 – +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm).

pH Dissolve 0.10 g of Spectinomycin Hydrochloride in 10 mL of water: the pH of the solution is between 4.0 and 5.6.

Water Not less than 16.0% and not more than 20.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Klebsiella pneumoniae* ATCC 10031

(2) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Spectinomycin Hydrochloride, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Streptomycin Sulfate

硫酸ストレプトマイシン

Change to read except the structural formula and chemical name:

Streptomycin Sulfate contains not less than 740 μg (potency) per mg, calculated on the dried basis. The potency of Streptomycin Sulfate is expressed as mass (potency) of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Description Streptomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and very slightly soluble in ethanol (95).

Identification **(1)** Dissolve 50 mg of Streptomycin Sulfate in 5 mL of water, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 10 minutes: a purple color is developed.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (7 in 100) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at about 150°C for about 5 minutes: the principal spots from the sample solution and the standard solution show the same in color tone and *R*f value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests for sulfate.

Optical rotation $[\alpha]_D^{20}$: -79 – $-88°$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH The pH of a solution obtained by dissolving 2.0 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Streptomycin Sulfate in 5 mL of water is clear, and colorless or pale yellow.

(2) Heavy metals—Proceed with 2.0 g of Streptomycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Prepare the test solution with 2.0 g of Streptomycin Sulfate according to Method 3 and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve exactly 0.20 g of Streptomycin Sulfate in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), add a mixture of methanol and sulfuric acid (97:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve exactly 36 mg of D(+)-mannose in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), and add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and acetic acid (100) (2:1:1) to a distance of 13 to 15 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at 110°C for 5 minutes: the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying Not more than 5.0% (0.5 g, reduced pres-

sure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Streptomycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Streptomycin Sulfate, equivalent to about 20 mg (potency), dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Sulbenicillin Sodium

スルベニシリンナトリウム

Change to read except the structural formula and chemical name:

Sulbenicillin Sodium contains not less than 814 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbenicillin Sodium is expressed as mass (potency) of sulbenicillin ($C_{16}H_{18}N_2O_7S_2$: 414.45).

Description Sulbenicillin Sodium occurs as white to light yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Sulbenicillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Sulbenicillin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Test (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +167 – +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.20 g of Sulbenicillin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 2.5 g of Sulbenicillin Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulbenicillin Sodium in 15 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the each peak other than the two peaks of sulbenicillin is not more than 2.0%, and the total amount of the peaks other than the two peaks of sulbenicillin is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 ± 0.1 with sodium hydroxide TS, and add water to make 1000 mL. To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of the lately eluted peak of sulbenicillin is about 18 minutes.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak of sulbenicillin after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system

suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the resolution between the two peaks of sulbenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total areas of the two peaks of sulbenicillin is not more than 5.0%.

Water Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.4 to 6.6 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Sulbenicillin Sodium Reference Standard, equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a freezer, and use within 4 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Sulbenicillin Sodium, equivalent to about 50 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μg (potency) and 10 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Talampicillin Hydrochloride

塩酸タランピシリン

Change to read except the structural formula and chemical name:

Talampicillin Hydrochloride is the hydrochloride of ampicillin phthalidyl ester.

It contains not less than 600 μg (potency) per mg, calculated on the anhydrous basis. The potency of Talampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Talampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

Identification **(1)** To 1 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of sodium hydroxide TS, mix, allow to stand for 5 minutes, and add 2 mL of dilute sulfuric acid and 2 to 3 drops of 2,4-dinitrophenylhydrazine TS: an orange-yellow precipitate is formed.

(2) Determine the infrared absorption spectrum of Talampicillin Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Talampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Talampicillin Hydrochloride (1 in 300) add 1 mL of dilute nitric acid, and add silver nitrate TS: a white precipitate is formed.

Optical rotation $[\alpha]_D^{20}$: +151 – +171° (0.2 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity **(1)** Heavy metals—Proceed with 1.0 g of Talampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Talampicillin Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL, 2 mL and 3 mL of the sample solution, add ethanol (99.5) to each to make exactly 100 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, ethyl acetate, water and ethanol (95) (4:4:2:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (99.5) (1 in 500) on

the plate, and heat at 110°C for 5 minutes: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution (3), and the total of the amount of each spot other than the principal spot from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (1), (2) and (3), is not more than 5%.

(4) 2-Formylbenzoic acid—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-formylbenzoic acid in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and acetic acid (100) (4:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of 2,4-dinitrophenylhydrazine in diluted sulfuric acid (6 in 25) (1 in 500): the spot of 2-formylbenzoic acid obtained from the sample solution is not more intense than that obtained from the standard solution.

Water Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Talampicillin Hydrochloride and Talampicillin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution. The standard solution should be prepared before use. Pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS, and allow them to stand for exactly 15 minutes. Add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, allow them to stand for exactly 15 minutes, and titrate with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared. If necessary, add 0.2 to 0.5 mL of starch TS. Separately, pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add exactly 10 mL of 0.005 mol/L iodine VS, titrate with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared, and make any necessary correction. For this titration, add 0.2 to 0.5 mL of starch TS, if necessary. Calculate the amount (mL) of 0.005 mol/L iodine VS, V_T and V_S, consumed by the sample solution and the standard solution, respectively.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = W_s \times \frac{V_T}{V_S} \times 1000$$

W_S: Amount [mg (potency)] of Talampicillin Hydrochloride Reference Standard

Containers and storage Containers—Tight containers.

Tegafur

テガフール

Change the Description to read:

Description Tegafur occurs as a white, crystalline powder.

It is soluble in methanol and in acetone, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

Change the Purity (4) to read:

Purity

(4) Arsenic—Prepare the test solution in a platinum crucible with 1.0 g of Tegafur according to Method 4, incinerating by ignition between 750°C and 850°C, and perform the test (not more than 2 ppm).

Teicoplanin

テイコプラニン

Change the origin/limits of content to read:

Teicoplanin contains not less than 900 μg (potency) per mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin ($C_{72\text{-}89}H_{68\text{-}99}Cl_2N_{8\text{-}9}O_{28\text{-}33}$).

Change the Content ratio of the active principle to read:

Content ratio of the active principle Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and calculate the sum of peak areas of teicoplanin A_2 group, S_a, the sum of peak areas of teicoplanin A_3 group, S_b, and the sum of peak areas of other contents, S_c from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A_2 group, teicoplanin A_3 group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

The elution order of each content and the relative retention time of each content to the retention time of teicoplanin $A_{2\text{-}2}$ are shown in the following table.

Name of content	Elution order	Relative retention time
teicoplanin A_3 group		≦0.42
teicoplanin $A_{3\text{-}1}$	1	0.29
teicoplanin A_2 group		0.42<, ≦1.25
teicoplanin $A_{2\text{-}1}$	2	0.91
teicoplanin $A_{2\text{-}2}$	3	1.00
teicoplanin $A_{2\text{-}3}$	4	1.04
teicoplanin $A_{2\text{-}4}$	5	1.17
teicoplanin $A_{2\text{-}5}$	6	1.20
others		1.25<

Content ratio (%) of teicoplanin A_2 group

$$= \frac{S_a}{S_a + 0.83S_b + S_c} \times 100$$

Content ratio (%) of teicoplanin A_3 group

$$= \frac{0.83S_b}{S_a + 0.83S_b + S_c} \times 100$$

Content ratio (%) of others

$$= \frac{S_c}{S_a + 0.83S_b + S_c} \times 100$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Mobile phase B: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 550 mL of water, add 1400 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Flowing of the mobile phase: Flow mobile phase A for 10 minutes before injection. After injection, control the gradient by mixing the mobile A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 32	100 → 70	0 → 30
32 – 40	70 → 50	30 → 50
40 – 42	50 → 100	50 → 0

Flow rate: 1.8 mL per minute.

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin $A_{2\text{-}2}$ after the solvent peak.

System suitability—

Test for required detection: Confirm that peak height of teicoplanin $A_{2\text{-}2}$ obtained from the sample solution is equivalent to 90% of the full scale.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the symmetry coefficient of the peak of teicoplanin $A_{3\text{-}1}$ is not more than 2.2.

Change the Bacterial endotoxins to read:

Bacterial endotoxins Less than 0.75 EU/mg (potency).

Theophylline

テオフィリン

Change the Description to read:

Description Theophylline occurs as white crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Theophylline, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Delete the Purity (5).

Change the Assay to read:

Assay Weigh accurately about 0.25 g of Theophylline, previously dried, and dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture, and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.02 mg of $C_7H_8N_4O_2$

Thiamine Hydrochloride

塩酸チアミン

Change the Water to read:

Water Not more than 5.0% (30 mg, coulometric titration).

Thiamine Nitrate

硝酸チアミン

Change the Assay to read:

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Hydrochloride Reference Standard (previously determine its water content in the same manner as directed under Thiamine Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

$$\text{Amount (mg) of } C_{12}H_{17}N_5O_4S = W_S \times \frac{Q_T}{Q_S} \times 0.9706$$

W_S: Amount (mg) of Thiamine Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.1 g of sodium l-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of thiamine to that of the internal standard is not more than 1.0%.

Thiopental Sodium

チオペンタールナトリウム

Change the Purity (4) to read:

Purity

(4) Related substances—Dissolve 50 mg of Thiopental Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than those of thiopental in the sample solution is not larger than the peak area of thiopental in the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiopental is about 15 minutes.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of thiopental obtained from 20 μL of this solution is equivalent to 15 to 25% of that of thiopental obtained from 20 μL of the standard solution.

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak

area of thiopental is not more than 2.0%.

Tipepidine Hibenzate

ヒベンズ酸チペピジン

Change the Purity (4) to read:

Purity

(4) Related substances—(i) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the hibenzic acid and tipepidine from the sample solution is not larger than the peak area of the tipepidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of a solution of ammonium acetate (1 in 100) and tetrahydrofuran (32:13).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 12 minutes.

Time span of measurement: As long as the retention time of tipepidine after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 to 13% of that of tipepidine obtained from 20 μL of the standard solution.

System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with the resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 1.5%.

(ii) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total area of all peaks other than the area of the hibenzic acid and tipepidine from the sample solution is not larger than 1/2 times the peak area of the tipepidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and a solution of ammonium acetate (1 in 500) (13:7).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 10 minutes.

Time span of measurement: As long as the retention time of tipepidine after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μL of this solution is equivalent to 7 to 13% of that of tipepidine obtained from 20 μL of the standard solution.

System performance: Dissolve 12 mg of Tipepidine Hibenzate and 4 mg of xanthene in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, hibenzic acid, tipepidine and xanthene are eluted in this order with the resolution between the peaks of tipepidine and xanthene being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 3.0%.

Tobramycin

トブラマイシン

Change to read except the structural formula and chemical name:

Tobramycin contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Tobramycin is expressed as mass (potency) of tobramycin ($C_{18}H_{37}N_5O_9$).

Description Tobramycin occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Determine the spectrum of a solution of Tobramycin in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under the Nuclear Magnetic Resonance Spectroscopy (^{1}H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ5.1 ppm, a multiple signal B between δ2.6 ppm and δ4.0 ppm, and a multiple signal C between δ1.0 ppm and δ2.1 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 1:8:2.

(2) Dissolve 10 mg each of Tobramycin and Tobramycin Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 4 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia TS, 1-butanol and methanol (5:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100°C for 5 minutes: the *R*f values of the principal spots obtained from the sample solution and the standard solution are the same.

Optical rotation $[\alpha]_D^{20}$: +138 – +148° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.10 g of Tobramycin in 10 mL of water is between 9.5 and 11.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Tobramycin in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals—Proceed with 1.0 g of Tobramycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 80 mg of Tobramycin in 10 mL of diluted ammonia solution (28) (1 in 250), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia solution (28) (1 in 250) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), ethanol (95) and 2-butanone (1:1:1) to a distance of about 10 cm, air-dry the plate, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Water Not more than 11.0% (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination.

Residue on ignition Not more than 1.0% (0.5 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(3) Standard solutions—Weigh accurately an amount of Tobramycin Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Tobramycin, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Tocopherol

トコフェロール

Change the Assay to read:

Assay Dissolve about 50 mg each of Tocopherol and Tocopherol Reference Standard, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of tocopherol in the sample solution and the standard solution.

$$\text{Amount (mg) of } C_{29}H_{50}O_2 = W_S \times \frac{H_T}{H_S}$$

W_S: Amount (mg) of Tocopherol Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μL in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol is about 10 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol is not more than 0.8%.

Tocopherol Acetate

酢酸トコフェロール

Change the Assay to read:

Assay Dissolve 50 mg each of Tocopherol Acetate and Tocopherol Acetate Reference Standard, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of tocopherol acetate in the sample solution and the standard solution, respectively.

$$\text{Amount (mg) of } C_{31}H_{52}O_3 = W_S \times \frac{H_T}{H_S}$$

W_S: Amount (mg) of Tocopherol Acetate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol acetate is about 12 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol acetate is not more than 0.8%.

Triamcinolone

トリアムシノロン

Change the Assay to read:

Assay Dissolve about 20 mg each of Triamcinolone and Triamcinolone Reference Standard, previously dried and accurately weighed, in a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak height of triamcinolone to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{27}FO_6 = W_S \times \frac{Q_T}{Q_S}$$

W_S: Amount (mg) of Triamcinolone Reference Standard

Internal standard solution—Dissolve 15 mg of methyl parahydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone is about 10 minutes.

System suitability—

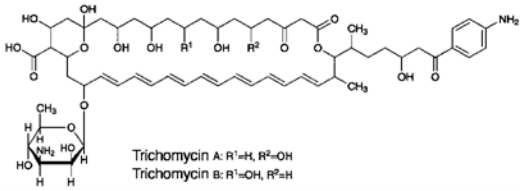
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, triamcinolone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone to that of the internal standard is not more than 1.5%.

Change to read:

Trichomycin

トリコマイシン



Trichomycin A
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,9,11,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [*12698-99-6*]
Trichomycin B
33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,7,9,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [*12699-00-2*]
[*1394-02-1*, Trichomycin]

Trichomycin contains not less than 7000 Units per mg, calculated on the dried basis. The potency of Trichomycin is expressed as unit based on the amount of trichomycin. 1 unit of Trichomycin is equivalent to 0.05 μg of trichomycin.

Description Trichomycin occurs as a yellow to yellow-brown powder.

It is practically insoluble in water, in ethanol (99.5) and in tetrahydrofuran.

It dissolves in dilute sodium hydroxide TS.

It is hygroscopic.

Identification **(1)** To 2 mg of Trichomycin add 2 mL of sulfuric acid: a blue color appears, and the color is changed to a blue-purple after allowing to stand.

(2) Dissolve 1 mg of Trichomycin in 50 mL of a solution of sodium hydroxide (1 in 200). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry: it exhibits maxima between 359 nm and 365 nm, between 378 nm and 384 nm, and between 400 nm and 406 nm.

Content ratio of the active principle Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve about 10 mg of Trichomycin in 50 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amount of trichomycin A and trichomycin B by the area percentage method: the amount of trichomycin A is between 20% and 40%, and that of trichomycin B is between 15% and 25%. The relative retention time of trichomycin B with respect to trichomycin A is about 1.2.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in a mixture of 600 mL of water and 400 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of trichomycin A is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of trichomycin A.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 30 mL. Confirm that the peak area of trichomycin A obtained from 5 μL of this solution is equivalent to 12 to 22% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, trichomycin A and trichomycin B are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichomycin A is not more than 2.0%.

Loss on drying Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately an amount of Trichomycin and Trichomycin Reference Standard, equivalent to about 150,000 units, dissolve them separately in a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of trichomycin.

$$\text{Amount (unit) of trichomycin} = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (unit) of Trichomycin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 15 g of ammonium acetate in 120 mL of water, and add 1000 mL of acetonitrile for liquid chromatography and 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of trichomycin is about 6 minutes.

System suitability—

System performance: Dissolve 5 mg of Trichomycin and 1 mg of berberine chloride in 100 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1). When the procedure is run with 20 μL of this solution under the above operating conditions, berberine and trichomycin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichomycin is not more than 2.0%.

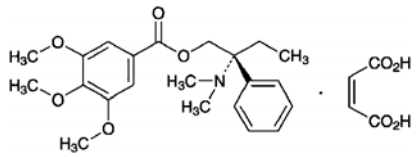
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Add the following:

Trimebutine Maleate

マレイン酸トリメブチン



and enantiomer

$C_{22}H_{29}NO_5.C_4H_4O_4$: 503.54

(2*RS*)-2-Dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate monomaleate [*34140-59-5*]

Trimebutine Maleate, when dried, contains not less than 98.5% and not more than 101.0% of trimebutine maleate ($C_{22}H_{29}NO_5.C_4H_4O_4$).

Description Trimebutine Maleate occurs as white, crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), soluble in acetonitrile, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of it in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimebutine Maleate as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point 131 – 135°C

Purity **(1)** Heavy metals—Proceed with 2.0 g of Trimebutine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 2.0 g of Trimebutine Maleate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Trimebutine Maleate in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and trimebutine from the sample solution is not more than 1/2 times the peak area of trimebutine from the standard solution, and the total area of the peaks other than maleic acid and trimebutine is not more than the peak area of trimebutine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 650 mL of diluted perchloric acid (17 in 20,000), previously adjusted the pH to 3.0 with a solution of ammonium acetate (1 in 1000), add 1 g of sodium 1-pentanesulfonate to dissolve. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of trimebutine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of trimebutine after the peak of maleic acid.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 20 mL. Confirm that the peak area of trimebutine obtained from 20 μL of this solution is equivalent to 20 to 30% of that from 20 μL of the standard solution.

System performance: Dissolve 40 mg of Trimebutine Maleate and 20 mg of imipramine hydrochloride in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7). When the procedure is run with 20μL of this solution under the above operating conditions, trimebutine and imipramine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5%.

Loss on drying Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.8 g of Trimebutine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.35 mg of $C_{22}H_{29}NO_5.C_4H_4O_4$

Containers and storage Containers—Well-closed containers.

Trimetoquinol Hydrochloride

塩酸トリメトキノール

Change the origin/limits of content to read:

Trimetoquinol Hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{19}H_{23}NO_5.HCl$ (mol. wt.: 381.85), calculated on the anhydrous basis.

Change the Description to read:

Description Trimetoquinol Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

Melting point: about 151°C (with decomposition, after drying in vacuum, 105°C, 4 hours).

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Trimetoquinol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetoquinol Hydrochloride as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetoquinol Hydrochloride (1 in 50) responds to the Qualitative Tests (1) for chloride.

Change the Optical rotation to read:

Optical rotation $[\alpha]_D^{20}$: −16 – −19° (0.25 g, calculated on the anhydrous basis, water, after warming and cooling, 25 mL, 100 mm).

Change the Purity to read:

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Trimetoquinol Hydrochloride in 10 mL of water by warming: the solution is clear and colorless.

(2) Sulfate—Perform the test with 0.5 g of Trimetoquinol Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals—Proceed with 1.0 g of Trimetoquinol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 50 mg of Trimetoquinol Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution, as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of trimetoquinol from the sample solution is not larger than the peak area of trimetoquinol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2 g of potassium dihydrogen phosphate and 2 g of sodium 1-pentane sulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH between 2.8 and 3.2, and filter through a membrane filter with pore size of 0.4 μm. Add 200 mL of acetonitrile to 800 mL of the filtrate.

Flow rate: Adjust the flow rate so that the retention time of trimetoquinol is about 7 minutes.

Time span of measurement: About twice as long as the retention time of trimetoquinol after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of trimetoquinol obtained from 20 μL of this solution is equivalent to 7 to 13% of that of trimetoquinol obtained from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Trimetoquinol Hydrochloride and 1 mg of procaine hydrochloride in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, procaine and trimetoquinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetoquinol is not more than 2.0%.

Delete the Loss on drying and add the following:

Water 3.5 – 5.5% (0.3 g, volumetric titration, direct titration).

Vancomycin Hydrochloride

塩酸バンコマイシン

Change to read except the structural formula and chemical name:

Vancomycin Hydrochloride contains not less than 1000 μg (potency) per mg, calculated on the anhydrous basis. The potency of Vancomycin Hydrochloride is expressed as mass (potency) of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25).

Description Vancomycin Hydrochloride occurs as a white powder.

It is freely soluble in water, soluble in formamide, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Vancomycin Hydrochloride (1 in 10,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vancomycin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vancomycin Hydrochloride as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Vancomycin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Vancomycin Hydrochloride in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation $[\alpha]_D^{20}$: -30 – $-40°$ (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH The pH of a solution obtained by dissolving 0.25 g of Vancomycin Hydrochloride in 5 mL of water is between 2.5 and 4.5.

Purity **(1)** Heavy metals—Proceed with 1.0 g of Vancomycin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. If necessary, proceed with 20 μL of the mobile phase A in the same manner to compensate for the base line. Determine each peak area by the automatic integration method: the area of each peak other than vancomycin from the sample solution is not more than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin is not more than 3 times of the peak area of vancomycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (92:7:1). Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (70:29:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (%)	Mobile phase B (%)
0 – 12	100	0
12 – 20	100 → 0	0 → 100
20 – 22	0	100

Flow rate: 1.5 mL per minute.

Time span of measurement: As long as about 2.5 times of

the retention time of vancomycin after the solvent peak.
System suitability—

Test for required detectability: Confirm that the peak area of vancomycin obtained from 20 μL of the standard solution is equivalent to 3 to 5% of that from 20 μL of the sample solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, heat at 65°C for 48 hours, and cool to the ordinal temperature. When the procedure is run with 20 μL of this solution under the above operating conditions, related substance 1, vancomycin and related substance 2 are eluted in this order, the resolution between the peaks of the related substance 1 and vancomycin is not less than 3, the theoretical plates of the peak of vancomycin is not less than 1500 steps, and the related substance 2 is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vancomycin is not more than 2.0%.

Water Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (3:1)).

Residue on ignition Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Bacillus subtilis* ATCC 6633

(2) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.2 to 6.4 after sterilization.

(3) Standard solutions—Weigh accurately an amount of Vancomycin Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Vancomycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Xylitol Injection

キシリトール注射液

Change the Containers and storage to read:

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

General Rules for Crude Drugs

Change the paragraph 1 to read:

1. Crude drugs in the monographs include medicinal parts obtained from plants or animals, cell inclusions and secretes separated from the origins, their extracts, and minerals. General Rules for Crude Drugs and the Crude Drugs in General Tests, Processes and Apparatus are applicable to the followings:

Acacia, Achyranthes Root, Agar, Agar Powder, Akebia Stem, Alisma Rhizome, Aloe, Alpinia Officinarum Rhizome, Amomum Seed, Anemarrhena Rhizome, Angelica Dahurica Root, Apricot Kernel, Areca, Artemisia Capillaris Flower, Asiasarum Root, Asparagus Tuber, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benzoin, Bitter Cardamon, Bitter Orange Peel, Bupleurum Root, Burdock Fruit, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Chrysanthemum Flower, Chuling, Cimicifuga Rhizome, Cinnamon Bark, Citrus Unshiu Peel, Clematis Root, Clove, Cnidium Rhizome, Coix Seed, Condurango, Coptis Rhizome, Corn Starch, Cornus Fruit, Corydalis Tuber, Cyperus Rhizome, Digenea, Digitalis, Dioscorea Rhizome, Ephedra Herb, Eucommia Bark, Evodia Fruit, Fennel, Forsythia Fruit, Fritillaria Bulb, Gambir, Gardenia Fruit, Gastrodia Tuber, Gentian, Geranium Herb, Ginger, Ginseng, Glehnia Root, Glycyrrhiza, Gypsum, Hemp Fruit, Hoelen, Honey, Houttuynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Jujube, Jujube Seed, Lithospermum Root, Longgu, Loquat Leaf, Magnolia Bark, Magnolia Flower, Mallotus Bark, Mentha Herb, Moutan Bark, Mulberry Bark, Notopterygium Rhizome, Nuphar Rhizome, Nux Vomica, Ophiopogon Tuber, Oriental Bezoar, Oyster Shell, Panax Rhizome, Peach Kernel, Peony Root, Perilla Herb, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Polygala Root, Polygonum Root, Potato Starch, Powdered Acacia, Powdered Alisma Rhizome, Powdered Aloe, Powdered Amomum Seed, Powdered Atractylodes Lancea Rhizome, Powdered Atractylodes Rhizome, Powdered Calumba, Powdered Capsicum, Powdered Cinnamon Bark, Powdered Clove, Powdered Cnidium Rhizome, Powdered Coix Seed, Powdered Coptis Rhizome, Powdered Cyperus Rhizome, Powdered Digitalis, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Hoelen, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Magnolia Bark, Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polypourus Sclerotium, Powdered Rhubarb, Powdered Rose Fruit, Powdered Scutellaria Root, Powdered Senega, Powdered Senna Leaf, Powdered Smilax Rhizome, Powdered Sophora Root, Powdered Sweet Hydrangea Leaf, Powdered Swertia Herb, Powdered Tragacanth, Powdered Zanthoxylum Fruit, Prunella Spike, Pueraria Root, Red Ginseng, Rehmannia Root, Rhubarb, Rice Starch, Rose Fruit, Rosin, Safflower, Saffron, Saposhnikovia Root, Saussurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sinomenium Stem, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Toad Venom, Tragacanth, Trichosanthes Root, Uncaria Thorn, Wheat Starch, Zanthoxylum Fruit, Zedoary.

Official Monographs for Part II

Acacia

アラビアゴム

Change the Identification to read:

Identification To 1 g of powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.01 g of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 10 μL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the *R*f value, respectively.

Change the Purity to read:

Purity **(1)** Insoluble residue—To 5.0 g of pulverized Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 0.01 g of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the *R*f value corresponding to glucose from the standard solution does not appear from the sample solution.

Powdered Acacia

アラビアゴム末

Change the Identification to read:

Identification To 1 g of Powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.01 g of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 10 μL each of the sample solution and the standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the *R*f value, respectively.

Change the Purity to read:

Purity **(1)** Insoluble residue—To 5.0 g of Powdered Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Powdered Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 0.01 g of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the *R*f value corresponding to glucose from the standard solution does not appear from the sample solution.

Aloe

アロエ

Change the origin/limits of content to read:

Aloe is the dried juice of the leaves mainly of *Aloe ferox* Miller, or of hybrids of the species with *Aloe africana* Miller or *Aloe spicata* Baker (*Liliaceae*).

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Change the Loss on drying to read:

Loss on drying Not more than 12.0%.

Add the following next to Extract content:

Component determination Weigh accurately about 0.1 g of pulverized Aloe, add 40 mL of methanol, and heat under a reflex condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of barbaloin for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 0.04 g of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and measure the peak areas of barbaloin, A_T and A_S, of both solutions.

$$\text{Amount (mg) of barbaloin} = W_S \times \frac{A_T}{A_S} \times 2$$

W_S: Amount (mg) of barbaloin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (74:26:1).

Flow rate: Adjust the flow rate so that the retention time of barbaloin is about 12 minutes.

System suitability—

System performance: To about 10 mg of barbaloin for component determination add 0.04 g of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add 1 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make exactly 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions except the wavelength of 300 nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloin is not more than 1.5%.

Powdered Aloe

アロエ末

Change the Identification (2) to read:

Identification (2) To 0.2 g of Powdered Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution has the same color tone and the same *R*f value with the red fluorescent spot from the standard solution.

Add the following:

Alpinia Officinarum Rhizome

Alpiniae officinari Rhizoma

リョウキョウ

Alpinia Officinarum Rhizome is the rhizome of *Alpinia officinarum* Hance (*Zingiberaceae*).

Description Alpinia Officinarum Rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2 to 8 cm in length, 0.6 to 1.5 cm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is as same as that of stele.

Odor, characteristic; taste, extremely pungent.

Under a microscope, transverse section reveals epidermal cells often containing resin-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and

stele composed of parenchyma interspersed with oil cells; parenchymatous cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2- to 8-compound), ovate, oblong or narrowly ovate, 10 – 40 μm in diameter and with an eccentric navel.

Identification To 0.5 g of pulverized Alpinia Officinarum Rhizome add 5 mL of acetone, shake for 5 minutes, and filter. Perform the test with the filtrate as directed under the Thin-layer Chromatography. Spot 5 μL of the filtrate on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane, ethyl acetate and acetic acid (100) (12:8:1) to a distance of about 10 cm, and air-dry the plate: two yellow-brown spots appear at around *R*f 0.4 – 0.5.

Loss on drying Not more than 15.0% (6 hours).

Total ash Not more than 7.5%.

Acid-insoluble ash Not more than 1.5%.

Extract content Dilute ethanol-extract: not less than 14.0%.

Areca

ビンロウジ

Change the Identification to read:

Identification Weigh 3 g of pulverized Areca in a glass-stoppered centrifuge tube, and add 30 mL of diethyl ether and 5 mL of sodium hydroxide TS, stopper tightly, shake for 5 minutes, centrifuge, and separate the diethyl ether layer. Evaporate the diethyl ether on a water bath, dissolve the residue in 1.5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of arecoline hydrobromide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and acetic acid (100) (10:6:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine TS on the plate: one spot among the spots from the sample solution and a red-brown spot from the standard solution show the same color tone and the same *R*f value.

Asiasarum Root

サイシン

Change the Description to read:

Description Asiasarum Root is a nearly cylindrical rhizome with numerous thin and long roots, externally light brown to dark brown. The root, about 15 cm in length, about 0.1 cm in diameter, with shallow longitudinal wrinkles on the surface, and brittle. The rhizome, 2 – 4 cm in length, 0.2 – 0.3 cm in diameter, often branched, with longitudinal wrinkles on the surface; internode short; each node has several scars of petiole and peduncle, and several thin and long roots.

Odor, characteristic; taste, acrid, with some sensation of numbness on the tongue.

Change the Purity (1) to read:

Purity **(1)** Terrestrial part—Any terrestrial parts are not found.

Add the following next to Purity (2):

Purity

(3) Aristolochic acid I—To exactly 2.0 g of pulverized Asiasarum Root add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve exactly 1.0 mg of aristolochic acid I for crude drugs purity test in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography, according to the following conditions: the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample solution shows such a peak, repeat the test under different conditions to confirm that the peak in question is not aristolochic acid I.

Operating conditions—

Detector: An ultraviolet or visible absorption photometer (wavelength: 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 2 mL of phosphoric acid in water to make 1000 mL and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of aristolochic acid I is about 15 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add diluted methanol (3 in 4) to make exactly 10 mL. Confirm that the ratio, S/N, of the signal (S) and noise (N) of aristolochic acid I obtained from 20 μL of this solution is not less than 3. In this case, S means the peak height on the chromatogram not including noise obtained by drawing an average line of the detector output, and N is 1/2 of the difference between the maximum and minimum output signals of the baseline around the peak in the range of 20 times the width at half-height of the peak.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aristolochic acid I is not more than 5.0%.

Add the following:

Asparagus Tuber

Asparagi Tuber

テンモンドウ

Asparagus Tuber is the tuber of *Asparagus cochinchinensis* Merrill (*Liliaceae*), from which most of the cork layer is removed, usually, after being steamed.

Description Asparagus Tuber is a fusiform to cylindrical tuber, 5 to 15 cm in length, 0.5 to 2 cm in diameter; externally light yellow-brown to light brown, translucent and often with longitudinal wrinkles; flexible, or hard and easily broken in texture; fractured surface, grayish yellow, glossy and horny.

Odor, characteristic; taste, sweet at first, followed by a slightly bitter aftertaste.

Under a microscope, a transverse section of Asparagus Tuber reveals stone cells and bundles of them on outer layer of cortex; mucilaginous cells containing raphides of calcium oxalate in the parenchyma cells of cortex and central cylinder; no starch grains.

Identification To 1 g of coarsely cut Asparagus Tuber add 5 mL of a mixture of 1-butanol and water (40:7), shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (10:6:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 2 minutes: the spot of a red-brown at first then changes to brown color appears at around *R*f 0.4.

Loss on drying Not more than 18.0% (6 hours).

Total ash Not more than 3.0%.

Add the following:

Burdock Fruit

Arctii Fructus

ゴボウシ

Burdock Fruit is the fruit of *Arctium lappa* Linné (*Compositae*).

Description Burdock Fruit is slightly curved, long obovate achene, 5 to 7 mm in length, 2.0 to 3.2 mm in width, 0.8 to 1.5 mm in thickness; externally grayish brown to brown, with black spots; hollow about 1 mm in diameter at one broad end; flat, indistinct, longitudinal ridge at the other narrow end. 100 fruits weighing 1.0 to 1.5 g.

Practically odorless; taste, bitter and oily.

Under a microscope, transverse section reveals an exocarp of single-layered epidermal tissue, mesocarp of slightly sclerified parenchyma, and endocarp of a single layer of stone cells; seed coat composed of radially elongated, sclerified epidermis, and parenchyma several cells thick; parenchymatous cells of the mesocarp contain a brown substance; stone cells of endocarp contain solitary, discrete crystals of calcium oxalate; cotyledons with starch grains, oil drops, aleurone grains, and minute crystals of calcium oxalate.

Identification To 0.5 g of pulverized Burdock Fruit add 20 mL of methanol, shake for 10 minutes, filter, and use filtrate as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate and water (15:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at around *R*f 0.4.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 7.0%.

Acid-insoluble ash Not more than 1.0%.

Extract content Dilute ethanol-extract: not less than 15.0%.

Capsicum

トウガラシ

Delete the Extract content.

Powdered Capsicum

トウガラシ末

Delete the Extract content.

Add the following:

Chrysanthemum Flower

Chrysanthemi Flos

キクカ

Chrysanthemum Flower is the capitulum of 1) *Chrysanthemum morifolium* Ramatulle or 2) *Chrysanthemum indicum* Linné (*Compositae*).

Description **1)** Chrysanthemum Flower is capitulum, 15 to 40 mm in diameter; involucre consisting of 3 to 4 rows of involucral scales; the outer involucral scale linear to lanceolate, inner involucral scale narrow ovate to ovate; ligulate flowers are numerous, white to yellow; tubular flowers in small number, light yellow-brown; tubular flowers occasionally degenerate; outer surface of involucre green-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) Chrysanthemum Flower is capitulum, 3 to 10 mm in diameter; involucre consisting of 3 to 5 rows of involucral scales; the outer involucral scale linear to lanceolatae, inner involucral scale narrow ovate to ovate; ligulate flower is single, yellow to light yellow-brown; tubular flowers in numerous, light yellow-brown; outer surface of involucre yellow-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Identification To 1 g of pulverized Chrysanthemum Flower add 20 mL of methanol, shake for 10 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (25:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of several spots obtained from the sample solution has the same color tone and the same *R*f value with the dark green spot obtained from the standard solution.

Loss on drying Not more than 15.0% (6 hours).

Total ash Not more than 8.5%.

Acid-insoluble ash Not more than 1.0%.

Extract content Dilute ethanol-extract: not less than 33.0%.

Cinnamon Bark

ケイヒ

Change the Total ash to read:

Total ash Not more than 6.0%.

Powdered Cinnamon Bark

ケイヒ末

Change the Total ash to read:

Total ash Not more than 6.0%.

Add the following:

Clematis Root

Clematidis Radix

イレイセン

Clematis Root is the root and rhizome of *Clematis chinensis* Osbeck, *Clematis manshurica* Ruprecht, *or Clematis hexapetala* Pallas (*Ranunculaceae*).

Description Clematis Root consists of short rhizome and numerous slender roots. The root, 10 to 20 cm in length, 1 to 2 mm in diameter, externally brown to blackish brown, with fine longitudinal wrinkles, brittle. The cortex easily separable from central cylinder; root, grayish white to light brown in the transverse section, light grayish yellow to yellow in the central cylinder; under a magnifying glass, central cylinder almost round, slight 2 to 4 sinuses on xylem. The rhizome, 2 to 4 cm in length, 5 to 20 mm in diameter, externally light grayish brown to grayish brown; cortex peeled off and fibrous, often with rising node; apex having the residue of lignified stem.

Odor, slight; practically tasteless.

Under a microscope, transverse section of root reveals a uni-layered epidermis in the outermost layer; with exodermis lying just inside of the epidermis; cortex and stele divided by endodermis; cortex composed of parenchymatous tissue; xylem with 2 – 4 small concavities where phloem is present; parenchymatous cells contain both simple and 2- to 8-compound starch grains.

Identification **(1)** To 0.5 g of pulverized Clematis Root add 10 mL of water, and boil for 2 to 3 minutes. After cooling, shake vigorously: a lasting fine foam appears.

(2) To 0.5 g of pulverized Clematis Root add 3 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To the filtrate add 1 mL of sulfuric acid gently: a brown color appears at the zone of contact.

Loss on drying Not more than 13.0% (6 hours).

Total ash Not more than 8.5%.

Acid-insoluble ash Not more than 3.0%.

Extract content Dilute ethanol-extract: not less than 15.0%.

Cod Liver Oil

肝油

Change the Description to read:

Description Cod Liver Oil is a yellow to orange oily liquid. It has a characteristic, slightly fishy odor and a mild taste.

It is miscible with chloroform.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air or by light.

Coptis Rhizome

オウレン

Change the Identification (2) to read:

Identification

(2) To 0.5 g of pulverized Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the chromatogram with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution with yellow to yellow-green fluorescence show the same color tone and the same *R*f value.

Powdered Coptis Rhizome

オウレン末

Change the Identification (2) to read:

Identification

(2) To 0.5 g of Powdered Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the chromatogram with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution with yellow to yellow-green fluorescence show the same color tone and the same *R*f value.

Add the following:

Eucommia Bark

Eucommiae Cortex

トチュウ

Eucommia Bark is the bark of *Eucommia ulmoides* Oliver (*Eucommiaceae*).

Description Eucommia Bark is a semi-tubular or plate-like bark, 2 to 6 mm in thickness; externally pale grayish brown to grayish brown, and rough in texture, sometimes reddish-brown due to the cork layer falling off; internally dark violet, smooth and covered with a linear pattern that runs longitudinally, silk-like threads of gutta-percha (a thermoplastic rubber-like substance) appearing when broken.

Odor faint but distinctive; taste slightly sweet.

Under a microscope, transverse section reveals parenchymatous cells containing gutta-percha; phloem with stone-cell and fiber layers; rays in rows of 2 – 3 cells; calcium oxalate crystals absent.

Identification Put 1 g of pulverized Eucommia Bark in a glass-stoppered centrifuge tube, add 10 mL of water and 20 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer so obtained, evaporate the diethyl ether on a water bath, and add 1 mL of ethanol (99.5) to the residue: colloidal substances appear.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 8.0%.

Acid-insoluble ash Not more than 5.0%.

Extract content Dilute ethanol-extract: not less than 7.0%.

Evodia Fruit

ゴシュユ

Change the origin/limits of content to read:

Evodia Fruit is the fruit of *Evodia rutaecarpa* Bentham or *Evodia officinalis* Dode or *Evodia bodinieri* Dode (*Rutaceae*).

Add the following:

Fritillaria Bulb

Fritillariae Bulbus

バイモ

Fritillaria Bulb is the bulb of *Fritillaria verticillata* Willdenow var. *thunbergii* Baker (*Liliaceae*).

Description Fritillaria Bulb is a depressed spherical bulb, 2 to 3 cm in diameter, 1 to 2 cm in height, consisting of 2 thickened scaly leaves often separated; externally and internally white to light yellow-brown in color; inside base is in a slightly dark color; the bulb sprinkled with lime before drying is dusted with white powder; fractured surface, white in color and powdery.

Odor, slight and characteristic; taste, bitter.

Under the microscope, a transverse section reveals the outermost layer (epidermis) to be composed of a single layer of cells; numerous vascular bundles scattered throughout the parenchyma inside of the epidermis; parenchyma filled with starch grains; starch grains are mainly simple (rarely 2 – 3 composite), 5 – 50 μm in diameter, narrowly ovate to ovate or triangular to obovate, stratiform figure obvious; epidermal cells and parenchymatous cells near the vessels contain solitary crystals of calcium oxalate.

Identification Put 2 g of pulverized Fritillaria Bulb in a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake for 20 minutes, and centrifuge. Take the upper layer, add 20 g of anhydrous sodium sulfate to the layer, shake, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: two spots of a yellow-red color appear at around *R*f 0.4 and at around *R*f 0.6.

Loss on drying Not more than 16.0% (6 hours).

Total ash Not more than 6.5%.

Acid-insoluble ash Not more than 1.0%.

Extract content Dilute ethanol-extract: not less than 8.0%.

Gardenia Fruit

サンシシ

Change the origin/limits of content to read:

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*).

It contains not less than 3.0% of geniposide ($C_{17}H_{24}O_{10}$: 388.37), calculated on the basis of dried material.

Change the Identification (1) to read:

Identification (1) To 1.0 g of pulverized Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

Add the following next to Identification:

Loss on drying Not more than 13.0%.

Add the following next to Total ash:

Component determination Weigh accurately about 0.5 g of pulverized Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and perform as the same as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and measure the peak areas of geniposide, A_T and A_S, of both solutions.

$$\text{Amount (mg) of geniposide} = W_S \times \frac{A_T}{A_S} \times 2$$

W_S: Amount (mg) of geniposide for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for component determination and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Add the following:

Gastrodia Tuber

Gastrodiae Tuber

テンマ

Gastrodia Tuber is the steamed tuber of *Gastrodia elata* Blume (*Orchidaceae*).

Description Gastrodia Tuber is an irregularly curved and flattened cylindrical to flattened fusiform tuber, 5 to 15 cm in length, 2 to 5 cm in diameter, 1 to 2 cm in thickness; externally light yellow-brown to light yellowish white; with ring nodes, and irregular longitudinal wrinkles; hard in texture; fractured surface, dark brown to yellow-brown in color, with luster, horny and gluey.

Odor, characteristic; practically tasteless.

Under a microscope, a transverse section reveals parenchyma cells containing needle raphides of calcium oxalate; starch grain absent.

Identification To 1 g of pulverized Gastrodia Tuber add 5 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 1 minutes: a red-purple spot appears at around *R*f 0.4.

Loss on drying Not more than 16.0% (6 hours).

Total ash Not more than 4.0%.

Extract content Dilute ethanol-soluble extract: not less than 16.0%.

Glycyrrhiza

カンゾウ

Change the Identification to read:

Identification To 2 g of pulverized Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizinic Acid Reference Standard in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Powdered Glycyrrhiza

カンゾウ末

Change the Identification to read:

Identification To 2 g of Powdered Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizinic Acid Reference Standard in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the with these solutions test as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Add the following:

Hemp Fruit

Cannabis Fructus

マシニン

Hemp Fruit is the fruit of *Cannabis sativa* Linné (*Cannabidaceae*).

Description Hemp Fruit is a slightly compressed void fruit, 4 to 5 mm in length, 3 to 4 mm in diameter; externally grayish green to grayish brown; pointed at one end, a scar of gynophore at the other end, and crest lines on both sides; outer surface lustrous with white mesh-like pattern; slightly hard pericarp; seed, slightly green in color and internally has grayish white albumen; 100 fruits weighing 1.6 to 2.7 g.

Practically odorless, aromatic on chewing; taste, mild and oily.

Under a microscope, a transverse section reveals the exocarp to be a single-layered epidermis; mesocarp composed of parenchyma, a pigment cell layer and rows of short, small cells; endocarp made up of a layer of radially elongated stone cells; seed coat comprises a tubular cell layer and spongy tissue. Inside of the seed; exosperm consists of one layer of parenchymatous cells, endosperm of one to several layers of parenchymatous cells; most of the embryo composed of parenchyma, vascular bundles occurring in the center of hypocotyls and cotyledons; embryo parenchyma contains aleurone grains and oil drops.

Identification To 0.3 g of pulverized Hemp Fruit add 3 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: a dark blue-purple spot appears at around *R*f 0.6.

Purity Bract—Hemp Fruit does not contain bract.

Loss on drying Not more than 9.0% (6 hours).

Total ash Not more than 7.0%.

Acid-insoluble ash Not more than 2.0%.

Imperata Rhizome

ボウコン

Change the Identification to read:

Identification To 1 g of pulverized Imperata Rhizome add 20 mL of hexane, allow the mixture to stand for 30 minutes with occasional shaking, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 5 mL of acetic anhydride, place 0.5 mL of this solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to blue-purple color.

Powdered Japanese Gentian

リュウタン末

Change the Identification to read:

Identification To 0.5 g of Powdered Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Add the following:

Jujube Seed

Zizyphi Semen

サンソウニン

Jujube Seed is the seed of *Zizyphus jujuba* Miller var. *spinosa* (Bunge) Hu ex H. F. Chou (*Rhamnaceae*).

Description Jujube Seed is a compressed ovate to orbicular, lenticular seed, 5 to 9 mm in lengh, 4 to 6 mm in width, 2 to 3 mm in thickness, externally brown to dark red-brown, glossy; hilum at one end, charaza at the other end; seed coat sightly fiexible, covering, milky white endosperm and light yellow embryo. 100 seeds weighing 3.0 to 4.5 g.

Odor, slightly oily; taste, mild and slightly oily.

Under a microscope, transverse section reveals seed coat composed of an upper epidermis, parenchyma and lower epidermis; upper epidermal cells sclerified and elongated in radial direction; lower epidermis covered with cuticle; endosperm composed of parenchyma, containing aggregated crystals of calcium oxalate, aleurone grains and starch grains; cotyledons composed of parenchyma that contains aleurone grains, starch grains and oil drops.

Identification To 2 g of pulverized Jujube Seed add 10 mL of methanol, and heat under a reflux condenser for 10

minutes. After cooling, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot appears at around *R*f 0.3, which shows a yellow-green to grayish green color after spraying 1-naphthol-sulfuric acid TS on the plate and heating at 105°C for 5 minutes.

Purity Foreign substance—Jujube Seed contains not more than 1.0% of the endocarp and other foreign substances.

Loss on drying Not more than 11.0% (6 hours).

Total ash Not more than 5.0%.

Extract content Dilute ethanol-extract: not less than 9.0%.

Add the following:

Loquat Leaf

Eriobotryae Folium

ビワヨウ

Loquat Leaf is the leaf of *Eriobotrya japonica* Lindley (*Rosaceae*).

Description Loquat Leaf is an oblong to wide lanceolate leaf, 12 to 30 cm in length, 4 to 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with short petiole; occasionally being cut into strips 5 to 10 mm in shorter diameter and several cm in longer diameter; upper surface green to green-brown in color, lower surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the lower surface of the leaf.

Odor, slight; practically tasteless.

Under a microscope, a transverse section of Loquat Leaf reveals thick cuticle on both surfaces; palisade tissue, mostly 4 to 5 layers with several large cells without chloroplast; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fiber attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll; woolly hair, unicellular and curved, about 25 μm in thickness, and up to 1.5 mm in length.

Identification To 0.3 g of pulverized Loquat Leaf add 10 mL of methanol, warm on a water bath for 5 minutes with occasional shaking, cool, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile and water (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: a red-purple principal spot appears at around *R*f 0.5.

Loss on drying Not more than 15.0% (6 hours).

Total ash Not more than 10.0%.

Extract content Dilute ethanol-soluble extract: not less than 16.0%.

Magnolia Bark

コウボク

Change the Extract content to read:

Extract content Dilute ethanol-soluble extract: not less than 11.0%.

Powdered Magnolia Bark

コウボク末

Change the Extract content to read:

Extract content Dilute ethanol-soluble extract: not less than 11.0%.

Add the following:

Magnolia Flower

Magnoliae Flos

シンイ

Magnolia Flower is the flower bud of *Magnolia salicifolia* Maximowicz, *Magnolia kobus* De Candolle, *Magnolia biondii* Pampanini, *Magnolia sprengeri* Pampanini or *Magnolia denudata* Desrousseaux (*Magnoliaceae*).

Description Magnolia Flower is a fusiform flower bud, 15 to 45 mm in length, 6 to 20 mm in diameter of central part; often having ligneous peduncles on base; usually 3 bracts, externally with sparse hairs; brown to dark brown; or with dense hairs, grayish white to light yellow-brown, and the inner surface smooth and dark brown in color; interior perianth of 9 pieces or 12 pieces, same size or exterior three pieces are smaller; 50 to 100 stamens and numerous pistils. Brittle in texture.

Odor, characteristic; taste, acrid and slightly bitter.

Identification To 1 g of pulverized Magnolia Flower add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 20 μL of the sample solution on a plate of

silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-red spot appears at around *R*f 0.3.

Loss on drying Not more than 14.0% (6 hours).

Total ash Not more than 5.5%.

Acid-insoluble ash Not more than 1.5%.

Extract content Dilute ethanol-extract: not less than 13.0%.

Essential oil content Perform the test with 50.0 g of pulverized Magnolia Flower as directed in the Essential oil content under the Crude Drugs Test: the volume of essential oil is not less than 0.5 mL.

Morphine and Atropine Injection

モルヒネ・アトロピン注射液

Change the Description to read:

Description Morphine and Atropine Injection is a clear, colorless liquid.

It is gradually colored by light.

pH: 2.5 – 5.0

Change the Identification to read:

Identification To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of Morphine Hydrochloride in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of Atropine Sulfate Reference Standard in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution, the standard solution (1) and the standard solution (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same *R*f value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

Change the Assay to read:

Assay (1) Morphine hydrochloride—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of morphine to that of the internal standard.

$$\text{Amount (mg) of morphine hydrochloride } (C_{17}H_{19}NO_3.HCl.3H_2O) = W_S \times \frac{Q_T}{Q_S} \times 1.1679$$

W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of Etilefrine Hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) Atropine sulfate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate Reference Standard (separately determine its loss on drying in the same manner as directed under Atropine Sulfate), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution.

Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak areas of atropine to that of the internal standard.

Amount (mg) of atropine sulfate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$

$$= W_S \times \frac{Q_T}{Q_S} \times \frac{1}{25} \times 1.027$$

W_S: Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of Etilefrine Hydrochloride (1 in 12,500).

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

Mulberry Bark

ソウハクヒ

Change the Identification to read:

Identification Heat 1 g of pulverized Mulberry Bark with 20 mL of hexane under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 10 mL of acetic anhydride, place 0.5 mL of the solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

Add the following:

Notopterygium Rhizome

Notopterygii Rhizoma

キョウカツ

Notopterygium Rhizome is the rhizome and root of *Notopterygium incisum* Ting ex H. T. Chang or *Notopterygium forbesii* Boissieu (*Umbelliferae*).

Description Notopterygium Rhizome is slightly curved, cylindrical to conical, 3 to 10 cm in length, 5 to 20 mm in diameter; rhizome occasionally branched; externally yellow-brown to dark brown. The rhizome with nearly orbicular, hollowed stem scars at the apex, sometimes having short residue of stem; externally node rising, internode short; root scars in warty processes on the node; externally root has coarse longitudinal wrinkles and lateral root scars in warty processes; light and slightly brittle in texture, easy to break. The transverse section of the rhizome reveals numerous radial cracks; cortex yellow-brown to brown; xylem light yellow to light grayish yellow; pith grayish white to light brown. Under a magnifying glass, the rhizome reveals brown, fine points of resin canals in the cortex and pith.

Odor, characteristic; taste, slightly acid at first, followed by a slightly pungent and slightly numbing aftertaste.

Under a microscope, transverse section shows the outermost layer to be composed of a cork layer several to a dozen or so cells thick; collenchyma just inside of the cork layer; oil canals scattered in cortex, large ones more than 300 μm in diameter; intercellular space occurring in radial direction in cortex; oil canals scattered in pith, large ones more than 500 μm in diameter; parenchymatous cells contain simple and 2- to 3-compound starch grains.

Identification To 0.3 g of pulverized Notopterygium Rhizome add 3 mL of hexane in a glass-stoppered centrifuge tube, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 10 μL of the sample solution on a plate of octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of methanol and water (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at around *R*f 0.5, which shows a dark purple color under ultraviolet light (main wavelength: 254 nm).

Loss on drying Not more than 13.0% (6 hours).

Total ash Not more than 6.5%.

Acid-insoluble ash Not more than 1.5%.

Extract content Dilute ethanol-extract: not less than 20.0%.

Opium Alkaloids Hydrochlorides

塩酸アヘンアルカロイド

Change the Description to read:

Description Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

It is soluble in water, and slightly soluble in ethanol (95).

It is colored by light.

Change the Purity (1) to read:

Purity **(1)** Clarity and color of solution－Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance at 420 nm is not more than 0.20.

Powdered Panax Rhizome

チクセツニンジン末

Change the Identification to read:

Identification Shake 0.5 g of Powdered Panax Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of several spots obtained from the sample solution shows the same color tone and the same *R*f value with the purple-red spot from the standard solution.

Peony Root

シャクヤク

Change the Identification (2) to read:

Identification

(2) To 2 g of pulverized Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS upon the plate, and heat at 105°C for 5 minutes: one spot among the spots from the sample solution and the purple-red spot from the standard solution show the same color tone and the same *R*f value.

Change the Assay to read:

Assay Weigh accurately about 0.5 g of pulverized Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin Reference Standard (separately detarmine the water content), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Paeoniflorin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust the flow rate so that the retention time of paeoniflorin is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Powdered Peony Root

シャクヤク末

Change the Identification (2) to read:

Identification

(2) To 2 g of Powdered Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one spot among the spots from the sample solution and the purple spot from the standard solution show the same color tone and the same *R*f value.

Phellodendron Bark

オウバク

Change the Identification (2) to read:

Identification

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

Powdered Phellodendron Bark

オウバク末

Change the Identification (2) to read:

Identification

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

Compound Phellodendron Powder for Cataplasm

パップ用複方オウバク散

Change the Identification to read:

Identification Shake thoroughly 0.2 g of Compound Phellodendron Powder for Cataplasm with 5 mL of methanol, filter, and use the filtrate as the sample solution. Dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value (berberine).

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder

オウバク・タンナルビン・ビスマス散

Change the Identification (1) to read:

Identification **(1)** Shake thoroughly 0.1 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f

value (berberine).

Delete the following Monograph:

Phenovalin and Magnesium Oxide Powder

フェノバリン・マグネシア散

Add the following:

Polygonum Root

Polygoni Multiflori Radix

カシュウ

Polygonum Root is the root of *Polygonum multiflorum* Thunberg (*Polygonaceae*), often being cut into round slices.

Description Polygonum Root is nearly fusiform, 10 to 15 cm in length, 2 to 5 cm in diameter; externally red-brown to dark brown; roughly wrinkled; a cross section light red-brown or light grayish brown, with numerous abnormal vascular bundles scattering irregularly around the large vascular bundles near center; heavy and hard in texture.

Odor, slight and characteristic; taste, astringent and slightly bitter.

Under a microscope, transverse section reveals the outermost layer to be several cells thick and composed of cork; cork cells contain brown substances; cortex composed of parenchyma; abnormal vascular bundles, exhibiting a ring of cambium; xylem lies inside of the cambium, and phloem outside; fibers lie outside the phloem; central portion of root lignified; parenchymatous cells contain aggregated crystals of calcium oxalate, and both simple and 2- to 8-compound starch grains; navel of starch grain obvious.

Identification To 1 g of pulverized Polygonum Root add 10 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 2 mL of methanol, and use this as the sample solution. Perform the test with the sample solution as directed under the Thin-layer Chromatography. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water, methanol and acetic acid (100) (200:10:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a fluorescent bluish white spot appears at around *R*f 0.3.

Loss on drying Not more than 14.0% (6 hours).

Total ash Not more than 5.5%.

Extract content Dilute ethanol-extract: not less than 17.0%.

Pueraria Root

カッコン

Change the origin/limits of content to read:

Pueraria Root is the root of *Pueraria lobata* Ohwi (*Leguminosae*), from which periderm has been removed.

It contains not less than 2.0% of puerarin ($C_{21}H_{20}O_9$: 416.38), calculated on the basis of dried material.

Change the Identification to read:

Identification To 2 g of pulverized Pueraria Root add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Puerarin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a blue-white spot from the standard solution show the same color tone and the same *R*f value.

Add the following next to Total ash:

Assay Weigh accurately about 0.3 g of pulverized Pueraria Root, add 50 mL of diluted methanol (1 in 2), and heat under a reflex condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and perform as the same as above. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Puerarin Reference Standard (separately detarmine the water content), add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and measure the peak areas of puerarin, A_T and A_S, of each solution.

$$\text{Amount (mg) of puerarin } (C_{21}H_{20}O_9) = W_S \times \frac{A_T}{A_S}$$

W_S: Amount (mg) of Puerarin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of puerarin is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of puerarin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of puerarin is not more than 1.5%.

Rhubarb

ダイオウ

Change the Identification to read:

Identification To 2 g of pulverized Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 40 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R*f value.

Delete the Component determination and add the following:

Assay Weigh accurately about 0.5 g of pulverized Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = W_S \times \frac{A_T}{A_S} \times \frac{1}{4}$$

W_S: Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column about 4 to 6 mm in inside diameter and about 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A Reference Standard and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Powdered Rhubarb

ダイオウ末

Change the Identification to read:

Identification To 2 g of Powdered Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A

Reference Standarad in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 40 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R*f value.

Delete the Component determination and add the following:

Assay Weigh accurately about 0.5 g of Powdered Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = W_S \times \frac{A_T}{A_S} \times \frac{1}{4}$$

W_S: Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column about 4 to 6 mm in inside diameter and about 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A Reference Standard and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Scopolia Extract and Ethyl Aminobenzoate Powder

ロートエキス・アネスタミン散

Change the Identification (4) to read:

Identification

(4) Place 30 g of Scopolia Extract and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 100 mL of water, shake for 30 minutes, and filter immediately by suction through a glass filter (G3). Transfer the residue in the flask to the same glass filter with the filtrate, and filter the residue by suction while pressing vigorously the residue on the same glass filter. Place 75 mL of the filtrate in a 300-mL beaker, and add cautiously 10 mL of diluted sulfuric acid (1 in 3). Add 0.2 mL of bromocresol green TS to this solution, and add dilute sulfuric acid dropwise while shaking thoroughly, until the color of the solution changes from green to yellow-green. After cooling, place this solution in a separator, wash with two 25-mL portions of a mixture of hexane and diethyl ether (1:1) by shaking well, and place the water layer in another separator. Make slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, separate the diethyl ether layer, add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate Reference Standard and 1 mg of Scopolamine Hydrobromide Reference Standard in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution show the same color tone and the same *R*f value with each yellow-red spot from the standard solutions, respectively.

Delete the following Monographs:

Compound Scopolia Extract and Tannic Acid Ointment

複方ロートエキス・タンニン軟膏

Compound Scopolia Extract and Tannic Acid Suppositories

複方ロートエキス・タンニン坐剤

Scutellaria Root

オウゴン

Change the Identification (2) to read:

Identification

(2) To 2 g of pulverized Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in methanol (1 in 100) on the plate: one spot among the spots from the sample solution and a dark green spot from the standard solution show the same color tone and the same *R*f value.

Powdered Scutellaria Root

オウゴン末

Change the Identification (2) to read:

Identification

(2) To 2 g of Powdered Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in methanol (1 in 100) on the plate: one spot among the spots from the sample solution and dark green spot from the standard solution show the same color tone and the same *R*f value.

Senna Leaf

センナ

Change the origin/limits of content to read:

Senna Leaf is the leaflets of *Cassia angustifolia* Vahl or *Cassia acutifolia* Delile (*Leguminosae*).

It contains not less than 1.0% of total sennosides [sennoside A ($C_{42}H_{38}O_{20}$: 862.75) and sennoside B ($C_{42}H_{38}O_{20}$: 862.75)], calculated on the basis of dried material.

Change the Identification (2) to read:

Identification

(2) To 2 g of pulverized Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standarad in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test as directed under the Thin-layer Chromatography with the sample solution and the standard solution. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R*f value.

Delete the Component determination and add the following:

Assay Weigh accurately about 0.5 g of pulverized Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add diluted methanol (7 in 10) twice with a 10-mL portion, shake for 10 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh

accurately about 10 mg of Sennoside B Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa}, of sennoside A, and the peak areas, A_{Tb} and A_{Sb}, of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennosides.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = W_{Sa} \times \frac{A_{Ta}}{A_{Sa}} \times \frac{1}{4}$$

$$\text{Amount (mg) of sennoside B } (C_{42}H_{38}O_{20}) = W_{Sb} \times \frac{A_{Tb}}{A_{Sb}} \times \frac{1}{2}$$

W_{Sa}: Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

W_{Sb}: Amount (mg) of Sennoside B Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet aborption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Powdered Senna Leaf

センナ末

Change the origin/limits of content to read:

Powdered Senna Leaf is the powder of Senna Leaf.

It contains not less than 1.0% of total sennosides [sennoside A ($C_{42}H_{38}O_{20}$: 862.75) and sennoside B ($C_{42}H_{38}O_{20}$: 862.75)], calculated on the basis of dried material.

Change the Identification (2) to read:

Identification

(2) To 2 g of Powdered Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R*f value.

Delete the Component determination and add the following:

Assay Weigh accurately about 0.5 g of Powdered Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add diluted methanol (7 in 10) twice with a 10-mL portoin, shake for 10 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B Reference Standard, dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each

of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa}, of sennoside A, and the peak areas, A_{Tb} and A_{Sb}, of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennoside.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = W_{Sa} \times \frac{A_{Ta}}{A_{Sa}} \times \frac{1}{4}$$

$$\text{Amount (mg) of sennoside B } (C_{42}H_{38}O_{20}) = W_{Sb} \times \frac{A_{Tb}}{A_{Sb}} \times \frac{1}{2}$$

W_{Sa}: Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

W_{Sb}: Amount (mg) of Sennoside B Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Sinomenium Stem

ボウイ

Change the Total ash to read:

Total ash Not more than 7.0%.

Swertia Herb

センブリ

Change the Identification to read:

Identification To 2 g of pulverized Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin Refereance Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same *R*f value.

Powdered Swertia Herb

センブリ末

Change the Identification to read:

Identification To 2 g of Powdered Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same *R*f value.

Swertia and Sodium Bicarbonate Powder

センブリ・重曹散

Change the Identification (1) and (2) to read:

Identification **(1)** To 10 g of Swertia and Sodium Bicarbonate Powder add 10 mL of ethanol (95), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Swertiamarin Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 30 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Identification under Powdered Swertia Herb.

(2) To 0.5 g of Swertia and Sodium Bicarbonate Powder add 10 mL of water. After stirring, centrifuge the mixture with 500 revolutions per minute. Smear, using a small glass rod, the slide glass with a small amount of the precipitate, add 1 drop of a mixture of water and glycerin (1:1), and put a cover glass on it so that the tissue section spreads evenly without overlapping each other, taking precaution against inclusion of bubbles, and use this as the preparation for microscopic examination. If the precipitate separates into two layers, proceed with the upper layer in the same manner, and use as the preparation for microscopic examination. Heat the preparation for microscopic examination in a short time: the preparation reveals the yellow-green to yellow-brown, approximately spherical pollen grains with granular patterns under a microscope. The pollen grains are 25 – 34 μm in diameter.

Toad Venom

センソ

Change the Identification to read:

Identification To 1 g of pulverized Toad Venom add 10 mL of acetone, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of resibufogenin for thin-layer chromatography in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane and acetone (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of several spots obtained from the sample solution has the same color tone and the same *R*f value with the blue-green spot obtained from the standard solution.

Add the following:

Uncaria Thorn

Uncariae Uncis Cum Ramulus

チョウトウコウ

Uncaria Thorn is, usually, the prickle of *Uncaria rhynchophylla* Miquel, *Uncaria sinensis* Haviland or *Uncaria macrophylla* Wallich (*Rubiaceae*).

Uncaria Thorn contains not less than 0.03% of total rhynchophylline (rhynchophylline and hirstine), calculated on the dried basis.

Description Uncaria Thorn is uncinate prickle or short stem with opposite or single prickle; the prickle, 1 to 4 cm in length, curved and acuminate; externally red-brown to dark brown or yellow-brown, some one with hairs, the transverse section oblong to elliptical, light brown; stem thin and prismatic square to cylindrical, 2 to 5 mm in diameter, externally, red-brown to dark brown or yellow-brown; the transverse section, square to elliptical; the pith light brown, square to elliptical; hard in texture.

Odorless and practically tasteless.

Under a microscope, a transverse section of the prickle reveals vascular bundles in the cortex, unevenly distributed and arranged in a ring. Parenchyma cells in the secondary cortex containing sand crystals of calcium oxalate.

Identification To 1 g of pulverized Uncaria Thorn add 20 mL of methanol, boil under a reflux condenser on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, add 5 mL of dilute acetic acid to the residue, warm the mixture on a water bath for 1 minute, and filter after cooling. Spot 1 drop of the filtrate on a filter paper, air-dry, spray Dragendorff's TS for spraying on it, and allow to stand: a yellow-red color develops.

Loss on drying Not more than 12.0% (6 hours).

Total ash Not more than 4.0%.

Extract content Dilute ethanol-extract: not less than 8.5%.

Component determination Weigh accurately about 0.2 g of medium powdered Uncaria Thorn, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add a mixture of methanol and dilute acetic acid (7:3) twice with a 10-mL portion, proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for component determination, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL,

and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solution (1) and (2) as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_{Ta} and A_{Tb}, of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, A_S, of rhynchophylline from the standard solution (1).

$$\text{Amount (mg) of total rhynchophylline} = W_S \times \frac{A_{Ta} + 1.405A_{Tb}}{A_S} \times \frac{1}{20}$$

W_S: Amount (mg) of rhynchophylline for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in about 200 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL, and add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rhynchophylline is about 17 minutes.

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for component determination in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and reflux for 10 minutes or heat at about 50°C for 2 hours. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is appears in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of rhynchophylline is not more than 1.5%.

Change to read:

Vitamin A Oil

ビタミンA油

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils. It contains not less than 30,000 Vitamin A Units per g. It may contain suitable antioxidants.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of vitamin A.

Description Vitamin A Oil is a yellow to yellow-brown, clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.

It is decomposed upon exposure to air or light.

Identification Dissolve Vitamin A Oil, Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution, the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution has the same color tone and the same *R*f value with the blue spot obtained from the standard solution (1) or the standard solution (2).

Purity **(1)** Acid − Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl ether (1:1), boil gently for 10 minutes under a reflux condenser, cool, and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Rancidity—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.

Change to read:

Vitamin A Oil Capsules

Vitamin A Capsules

ビタミンA油力プセル

Vitamin A Oil Capsules contain not less than 90.0% and not more than 130.0% of the labeled Units of Vitamin A.

Method of preparation Prepare as directed under Capsules, using Vitamin A Oil.

Description The content of Vitamin A Oil Capsules conforms to the requirements of the Description under Vitamin A Oil.

Identification Proceed the test with the content of Vitamin A Oil Capsules as directed in the Identification under Vitamin A Oil.

Assay Weigh accurately 20 Vitamin A Oil Capsules, and open the capsules to take out the content. Wash the capsules well with a small amount of diethyl ether, allow the capsules to stand at ordinal temperature to vaporize the diethyl ether, and weigh accurately. Perform the test with the content as directed under the Vitamin A Assay, and calculate the units of vitamin A per capsule. Before applying Method 1-1, it is necessary to know which the sample is, retinol acetate or retinol palmitate.

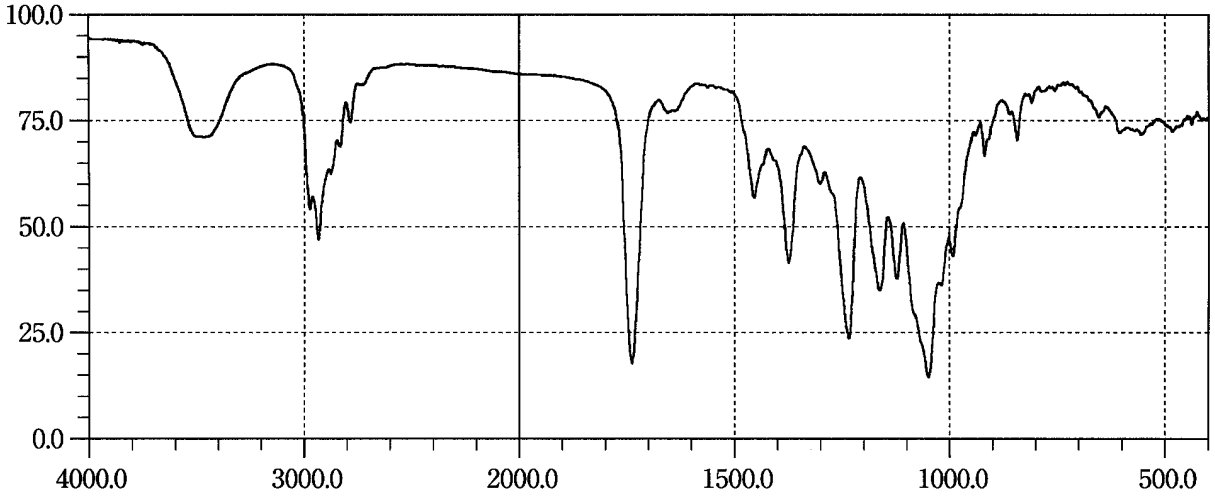
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

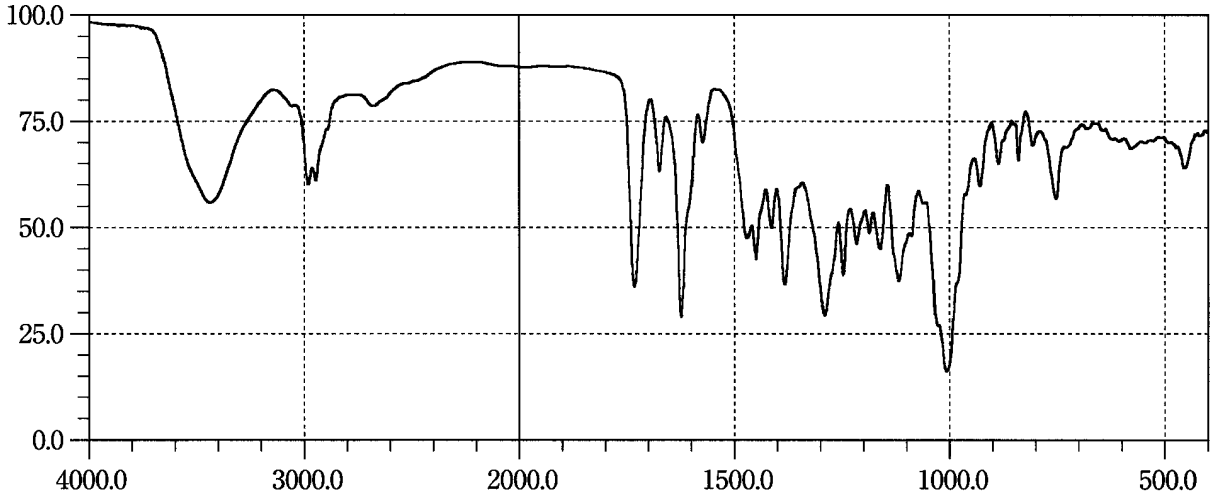
Part I

Acetylspiramycin



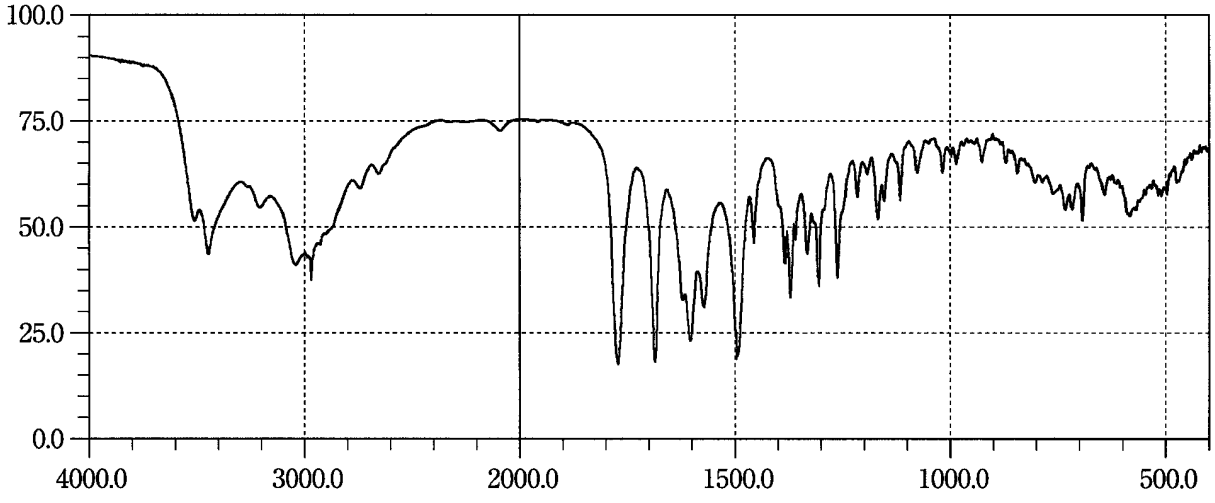
Preparation of sample: Potassium bromide disk method

Aclarubicin Hydrochloride



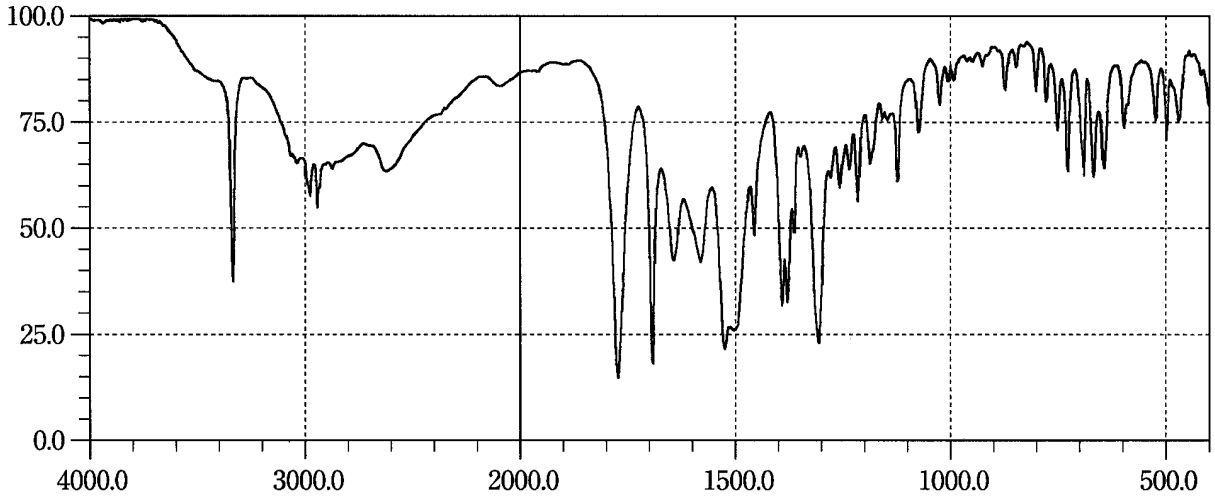
Preparation of sample: Potassium bromide disk method

Ampicillin



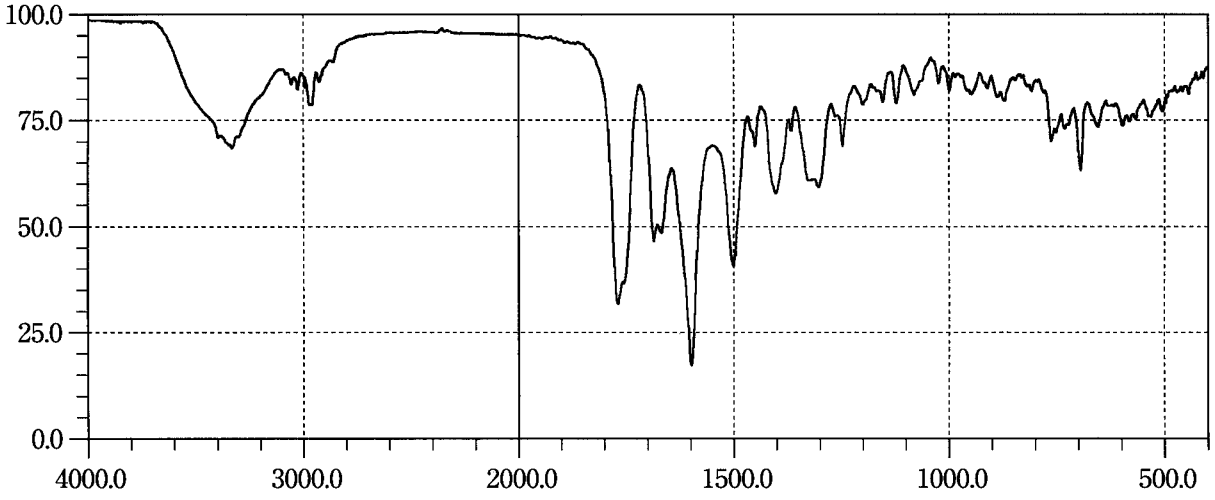
Preparation of sample: Potassium bromide disk method

Anhydrous Ampicillin



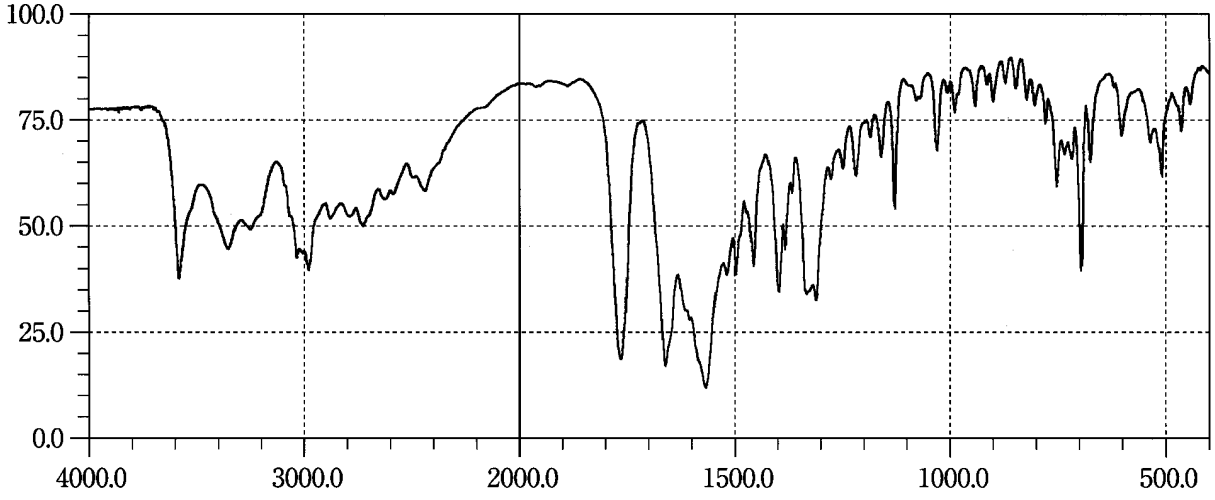
Preparation of sample: Potassium bromide disk method

Ampicillin Sodium



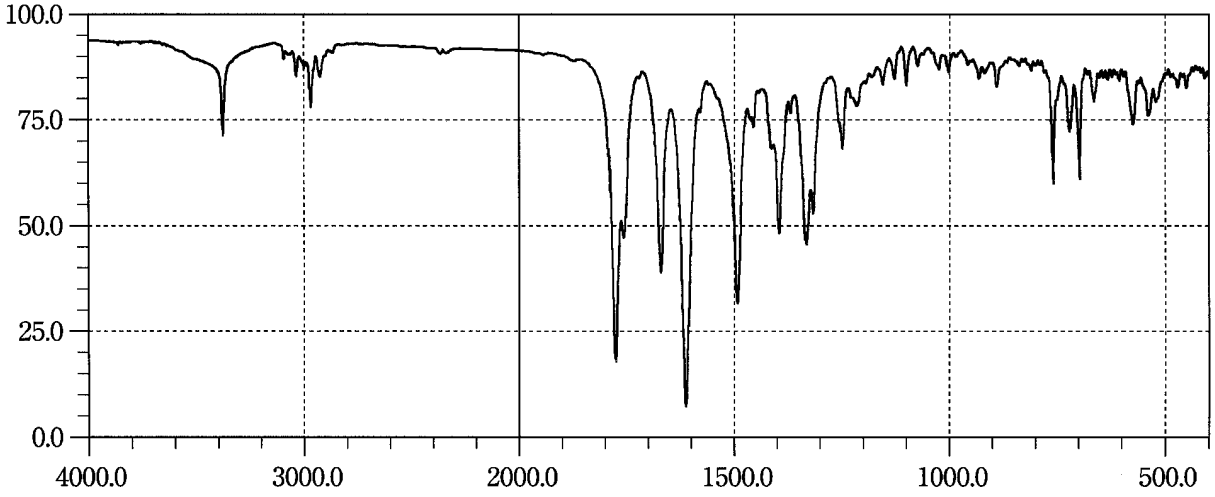
Preparation of sample: Potassium bromide disk method

Benzylpenicillin Benzathine



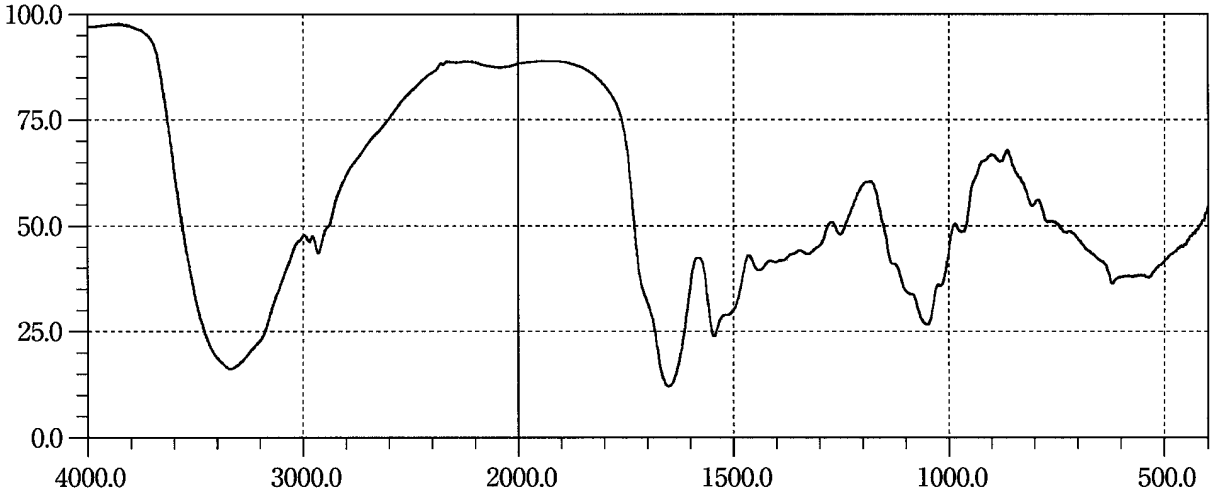
Preparation of sample: Potassium bromide disk method

Benzylpenicillin Potassium



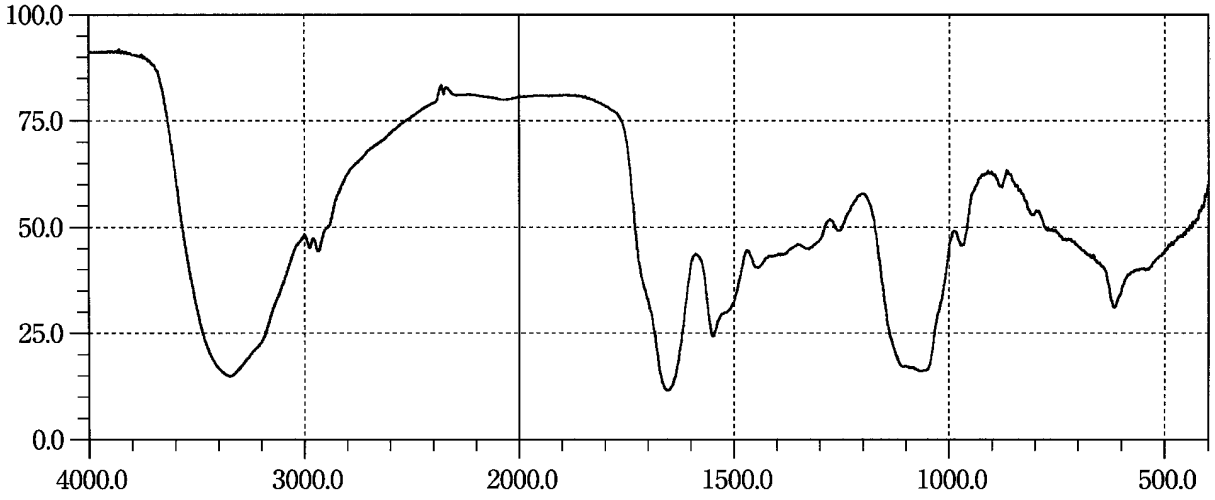
Preparation of sample: Potassium bromide disk method

Bleomycin Hydrochloride



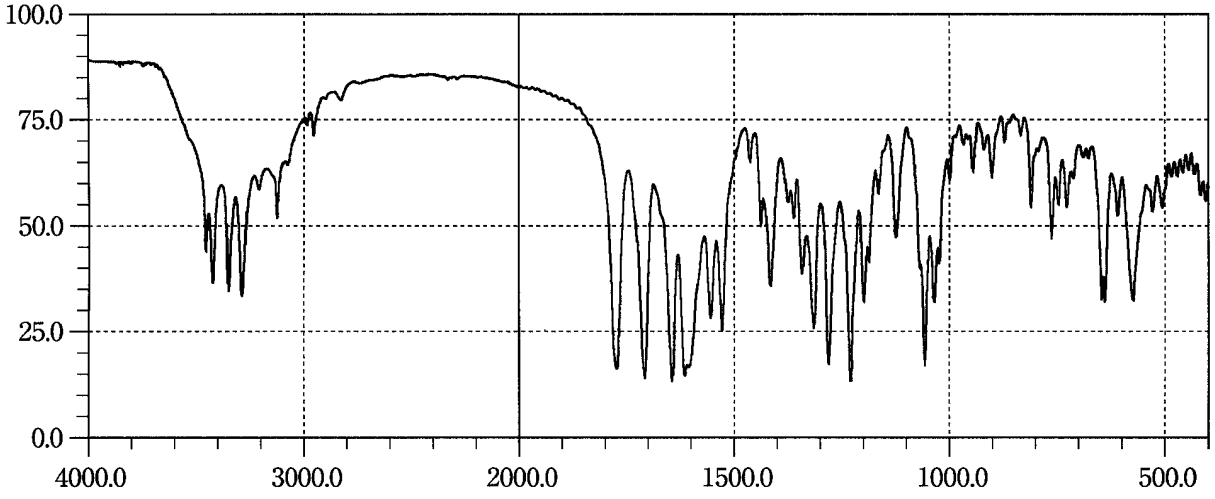
Preparation of sample: Potassium bromide disk method

Bleomycin Sulfate



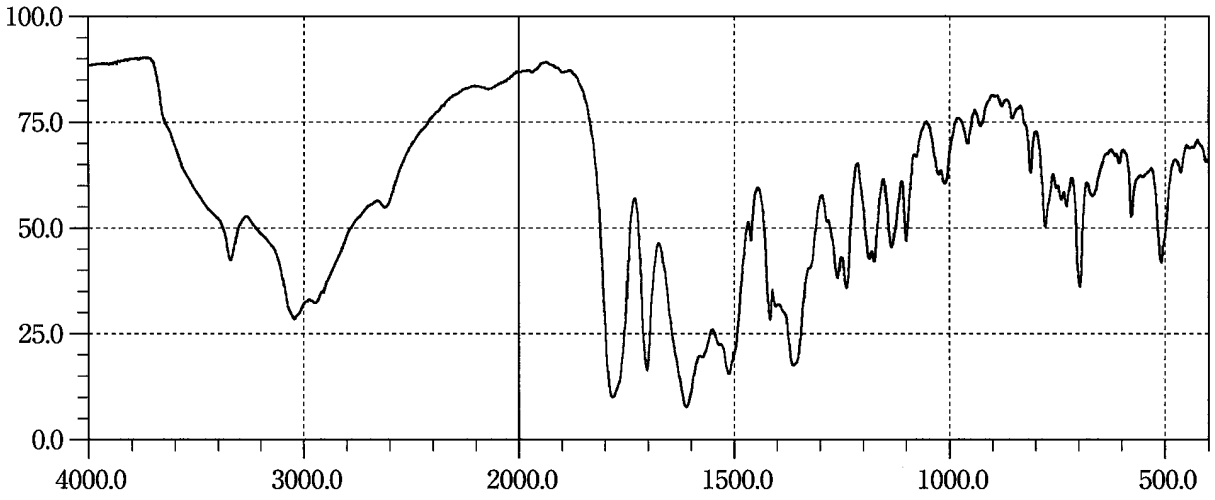
Preparation of sample: Potassium bromide disk method

Carumonam Sodium



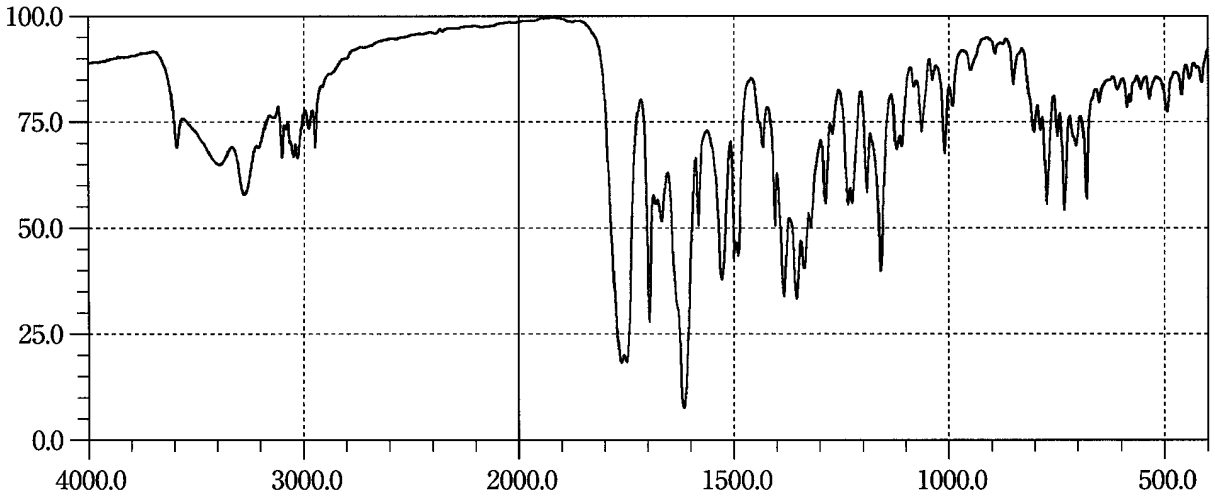
Preparation of sample: Potassium bromide disk method

Cefaclor



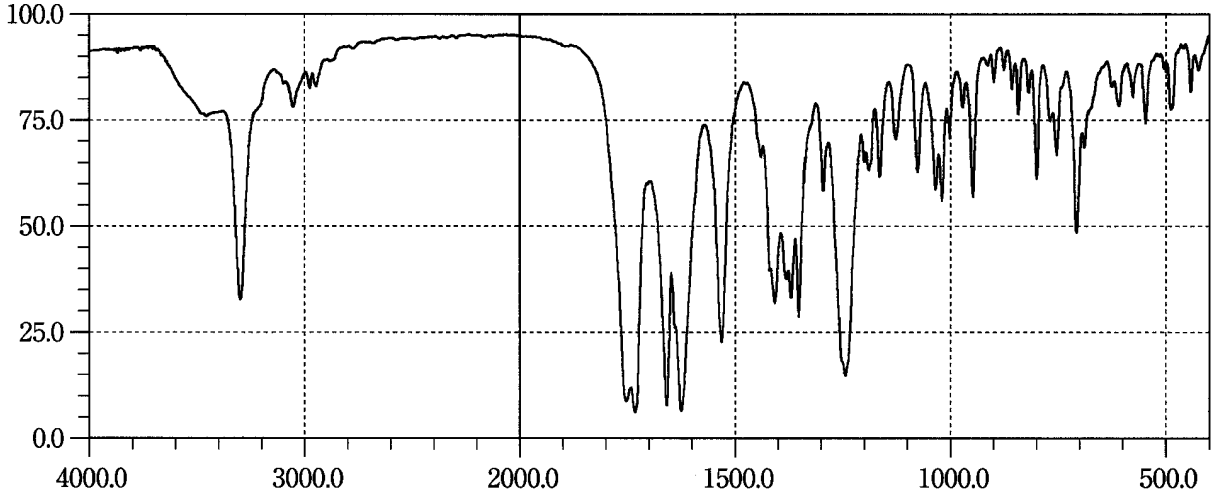
Preparation of sample: Potassium bromide disk method

Cefaloridin



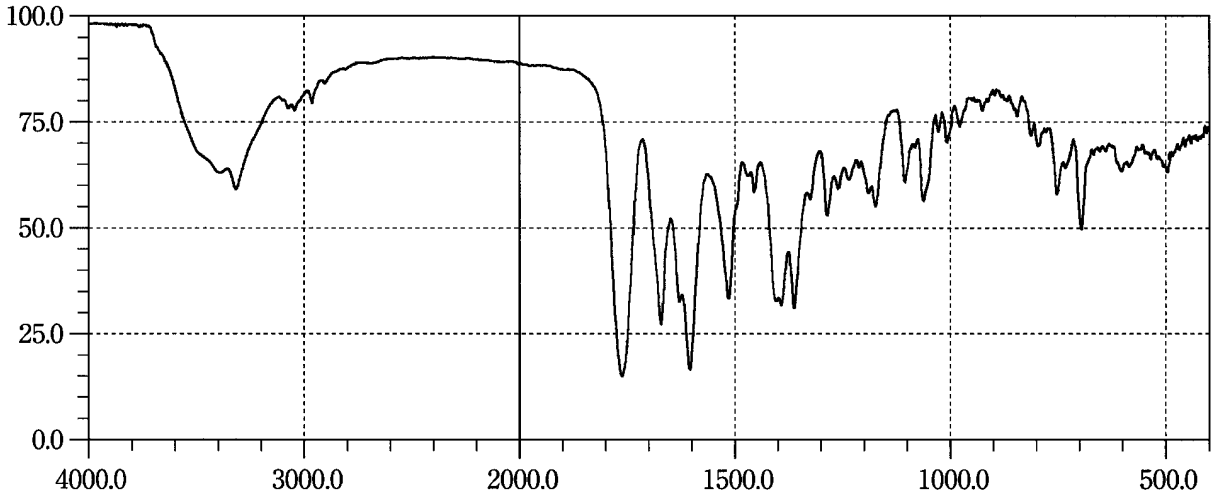
Preparation of sample: Potassium bromide disk method

Cefalotin Sodium



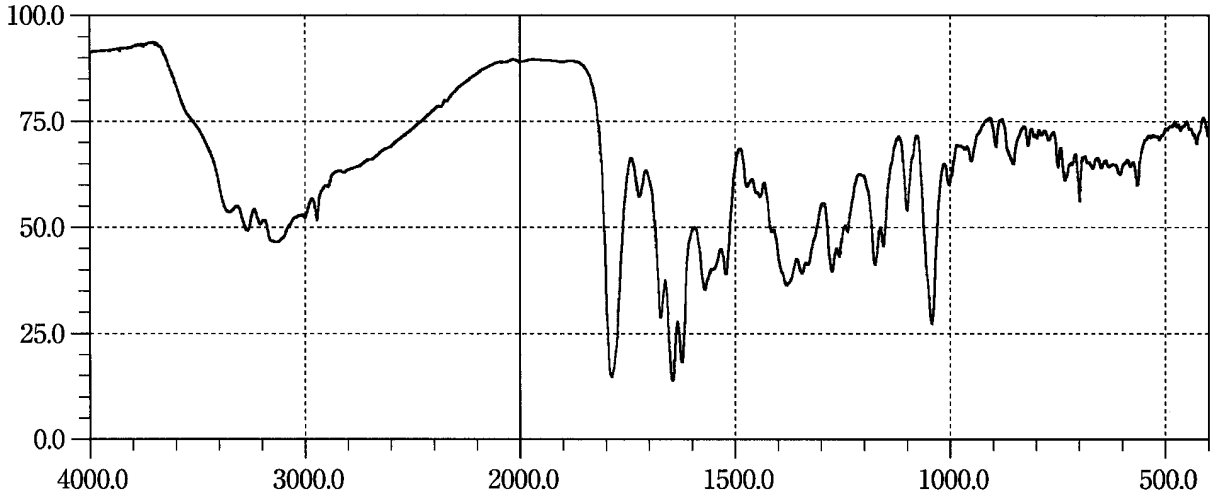
Preparation of sample: Potassium bromide disk method

Cefamandole Sodium



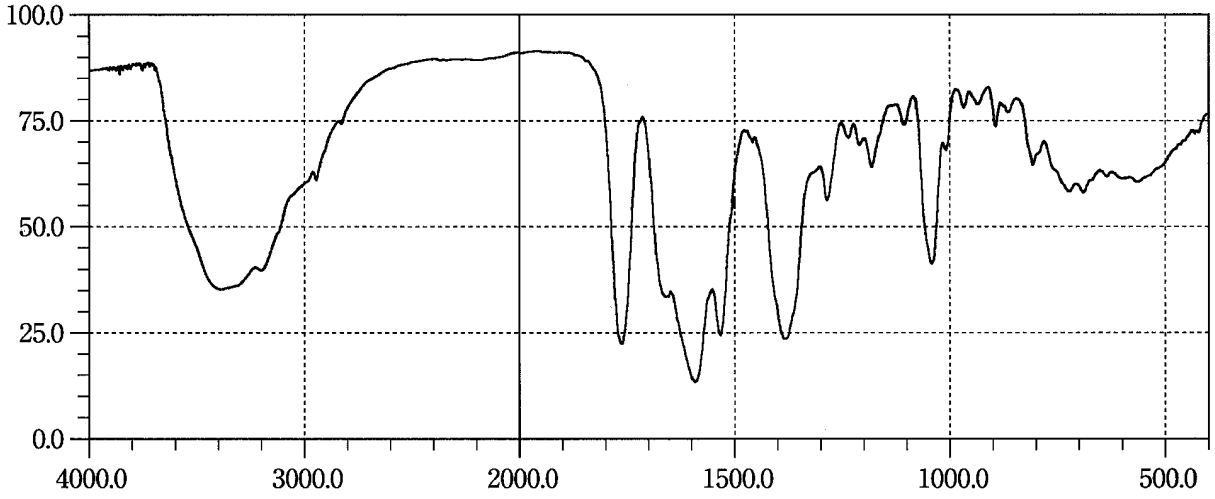
Preparation of sample: Potassium bromide disk method

Cefmenoxime Hydrochloride



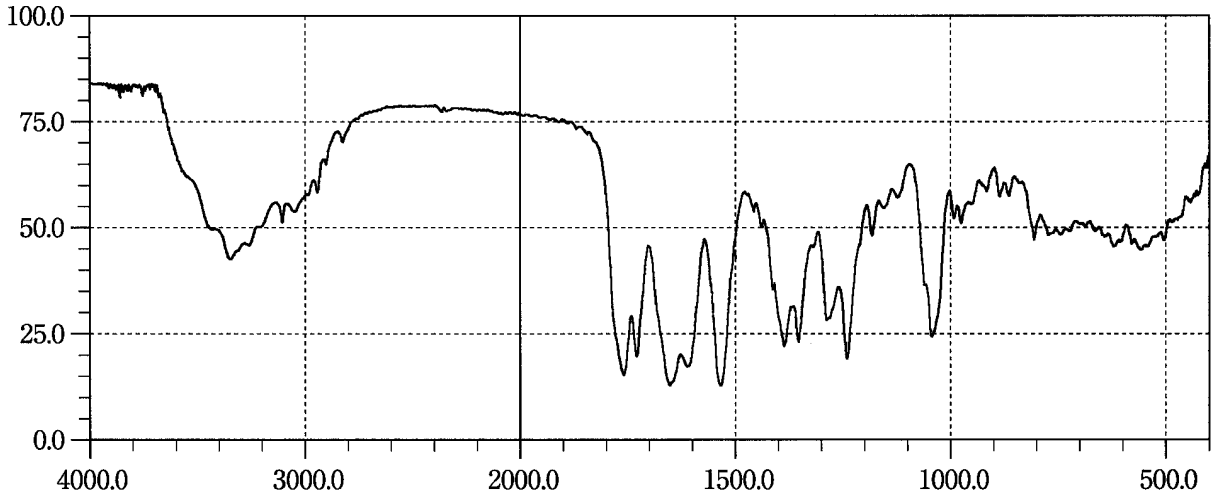
Preparation of sample: Potassium bromide disk method

Cefodizime Sodium



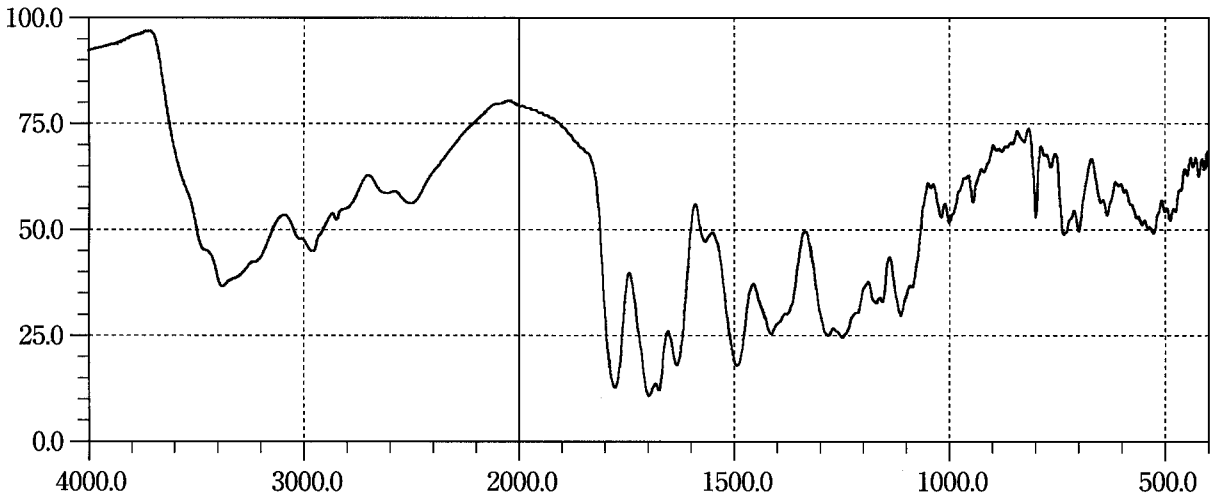
Preparation of sample: Potassium bromide disk method

Cefotaxime Sodium



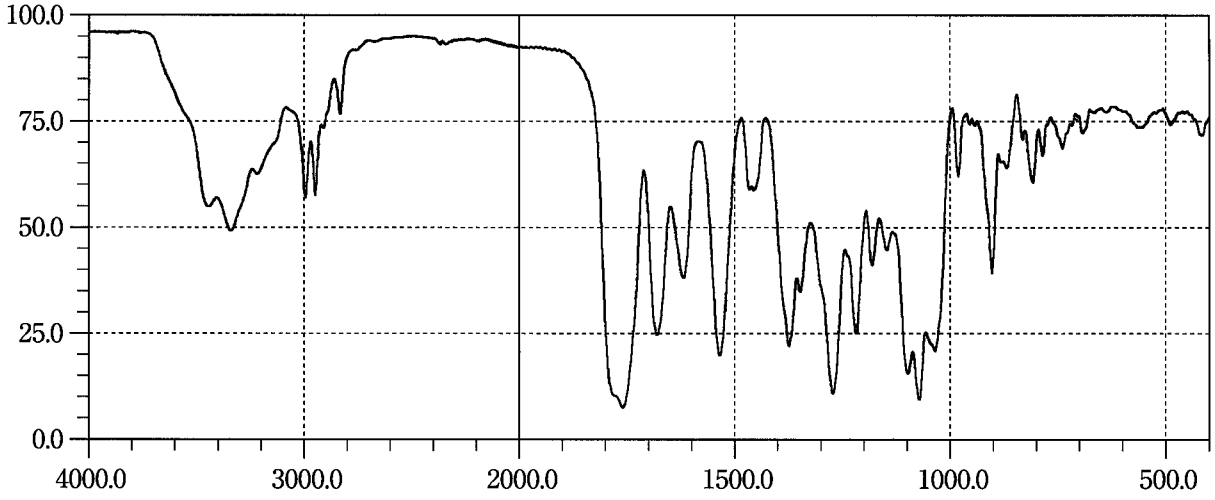
Preparation of sample: Potassium bromide disk method

Cefotetan



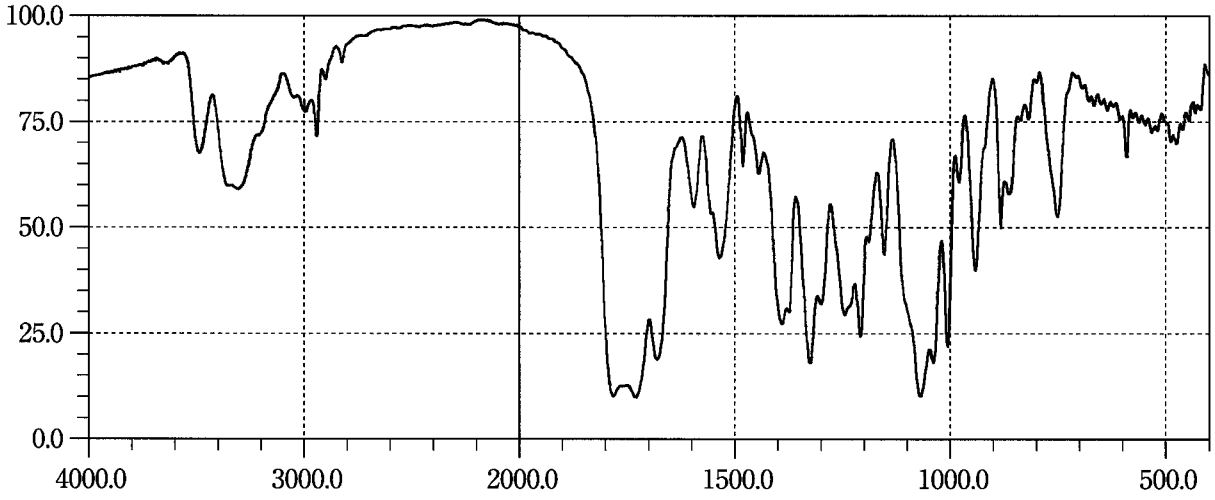
Preparation of sample: Potassium bromide disk method

Cefpodoxime Proxetil



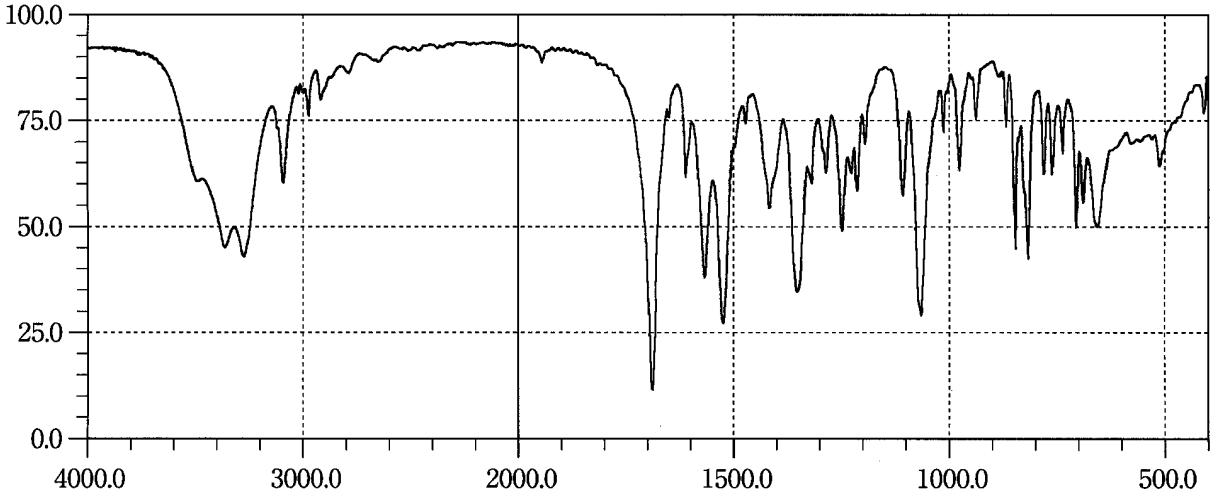
Preparation of sample: Potassium bromide disk method

Cefuroxime Axetil



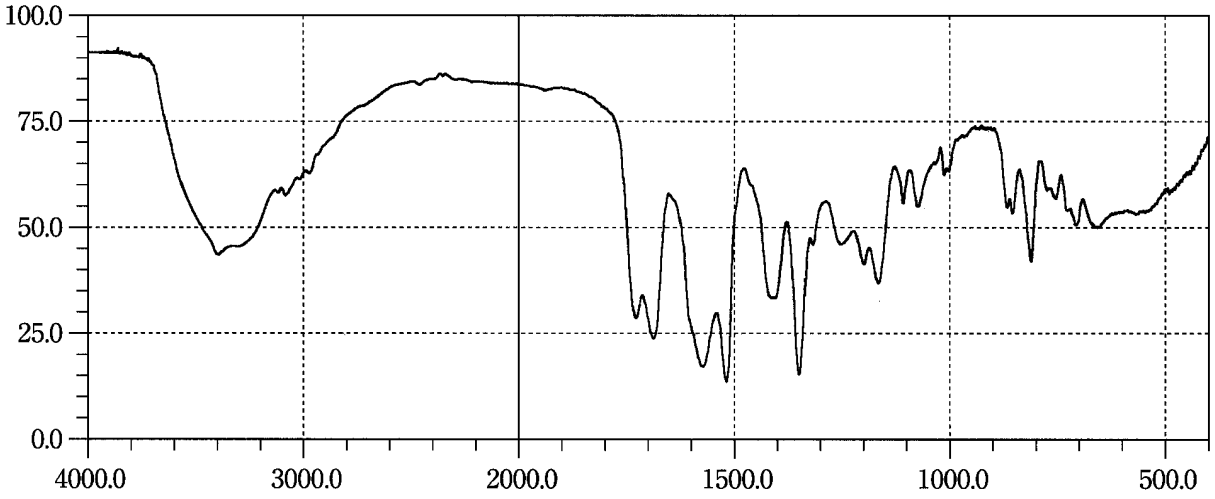
Preparation of sample: Potassium bromide disk method

Chloramphenicol



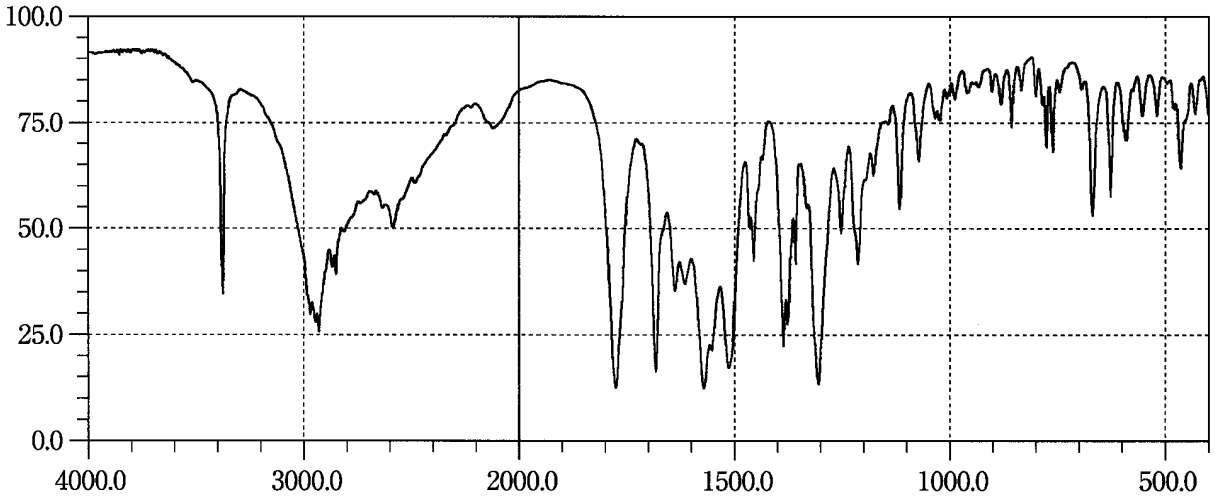
Preparation of sample: Potassium bromide disk method

Chloramphenicol Sodium Succinate



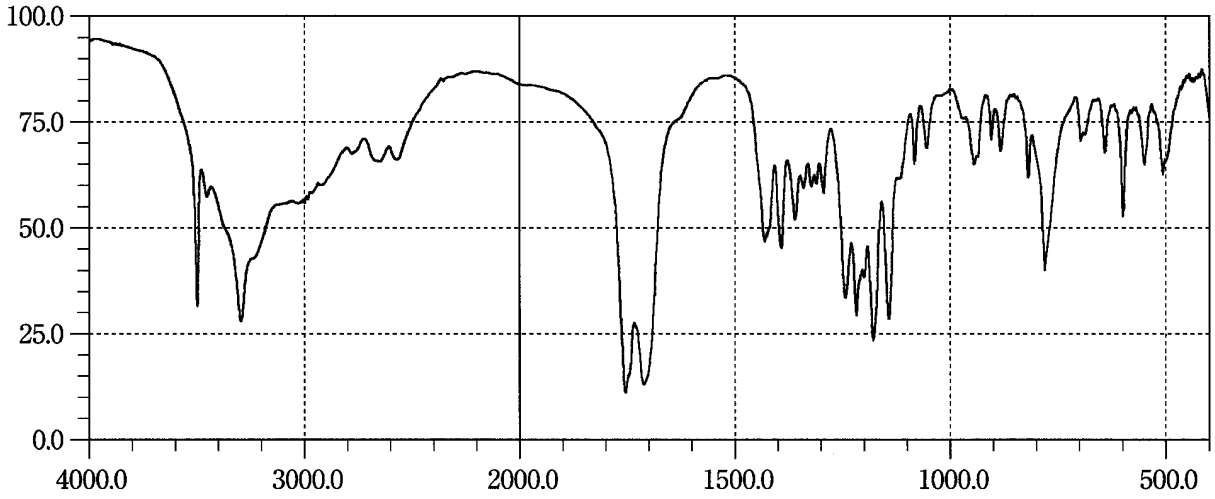
Preparation of sample: Potassium bromide disk method

Ciclacillin



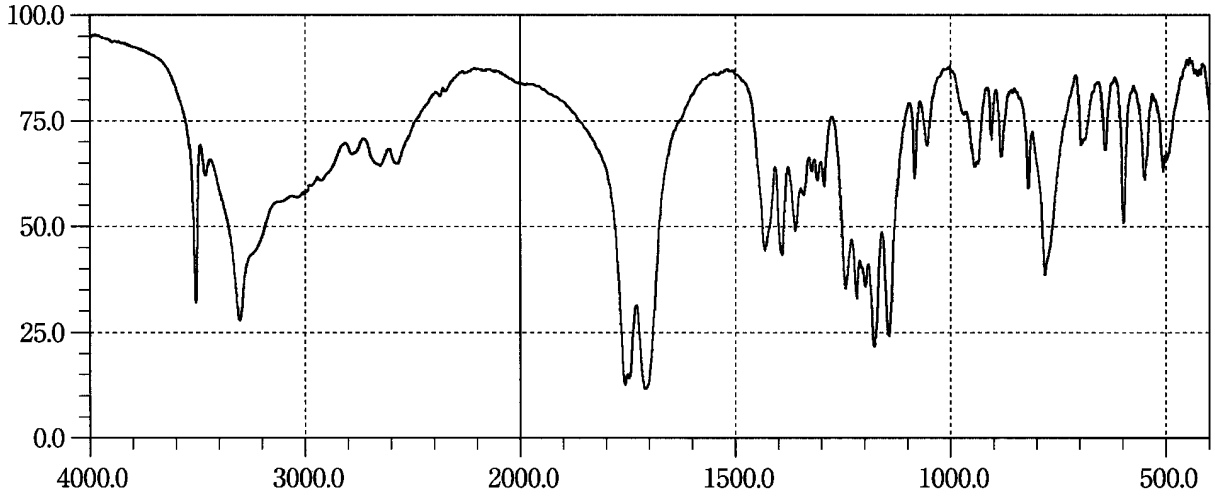
Preparation of sample: Potassium bromide disk method

Citric Acid



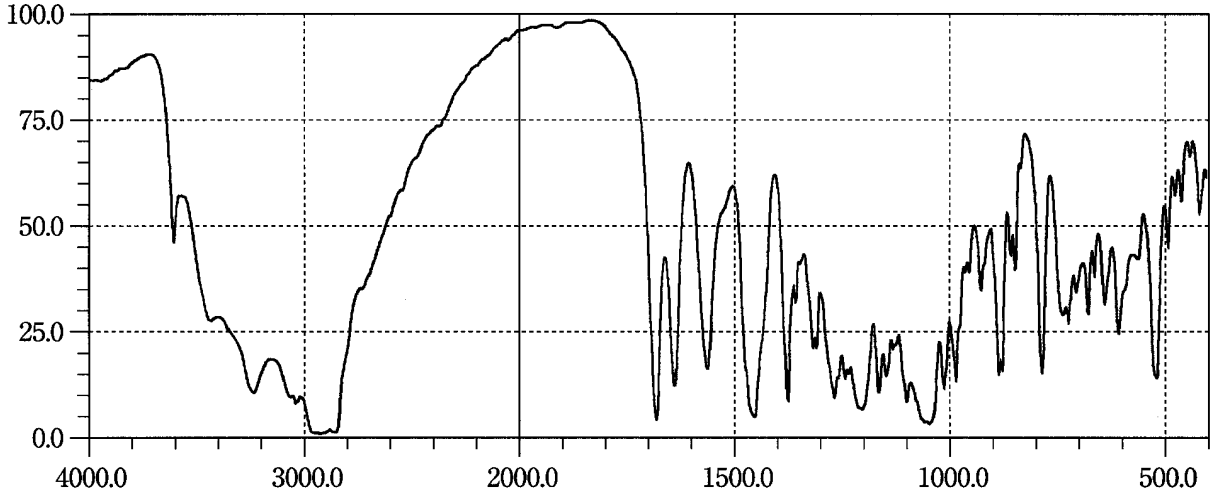
Preparation of sample: Potassium bromide disk method

Anhydrous Citric Acid



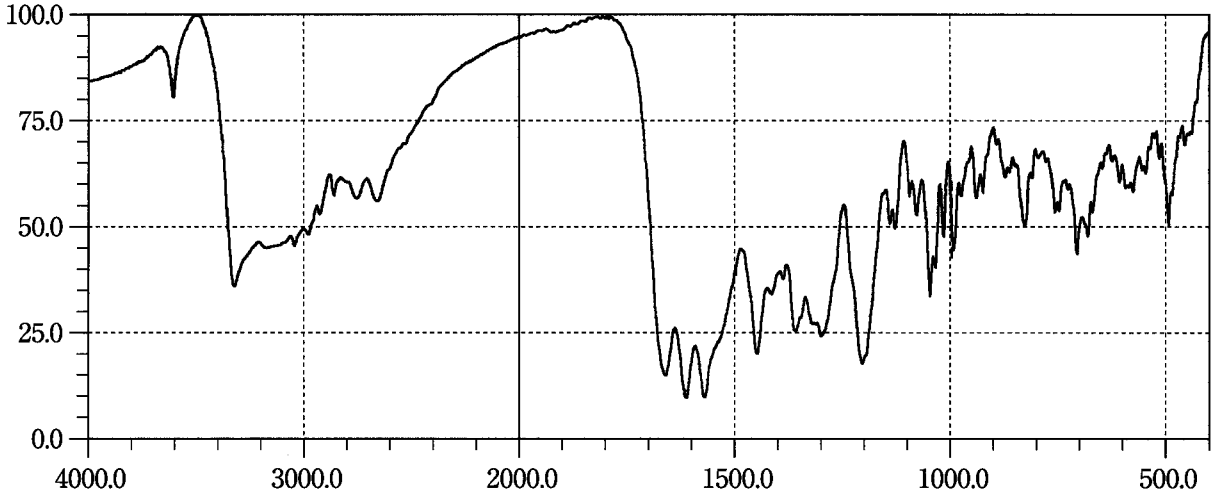
Preparation of sample: Potassium bromide disk method

Clindamycin Phosphate



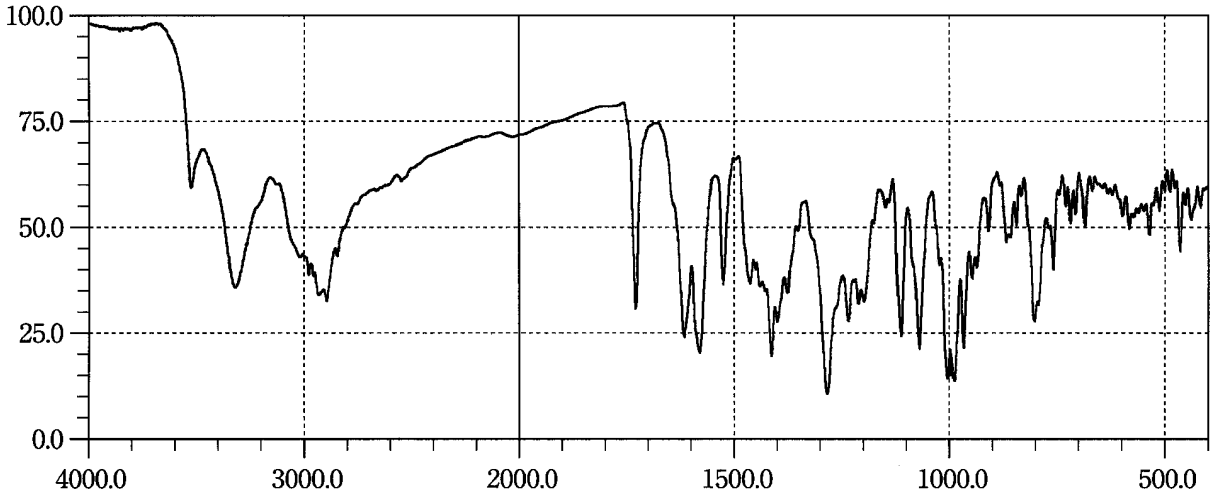
Preparation of sample: Paste method

Demethylchlortetracycline Hydrochloride



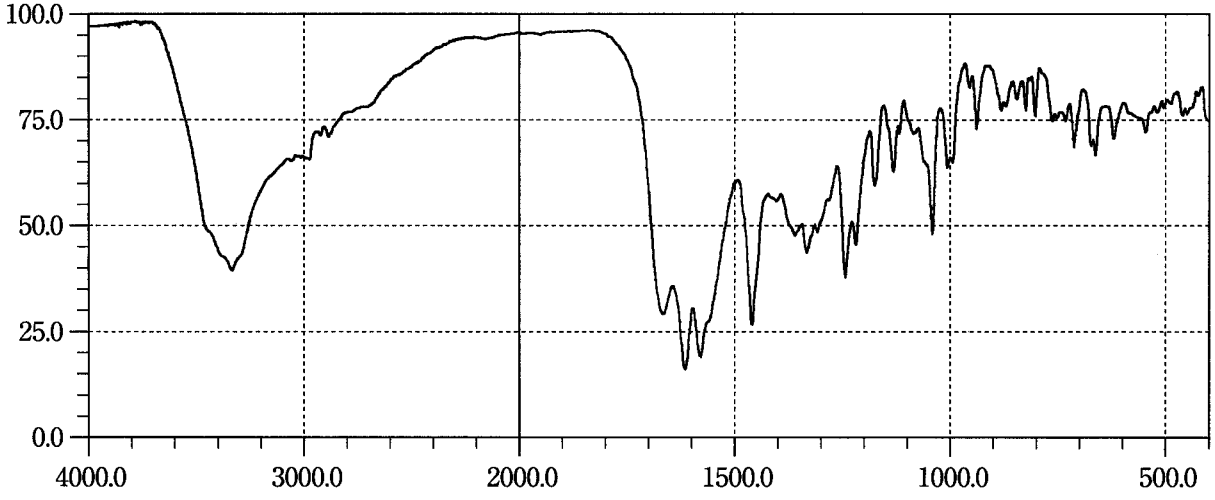
Preparation of sample: Potassium chloride disk method

Doxorubicin Hydrochloride



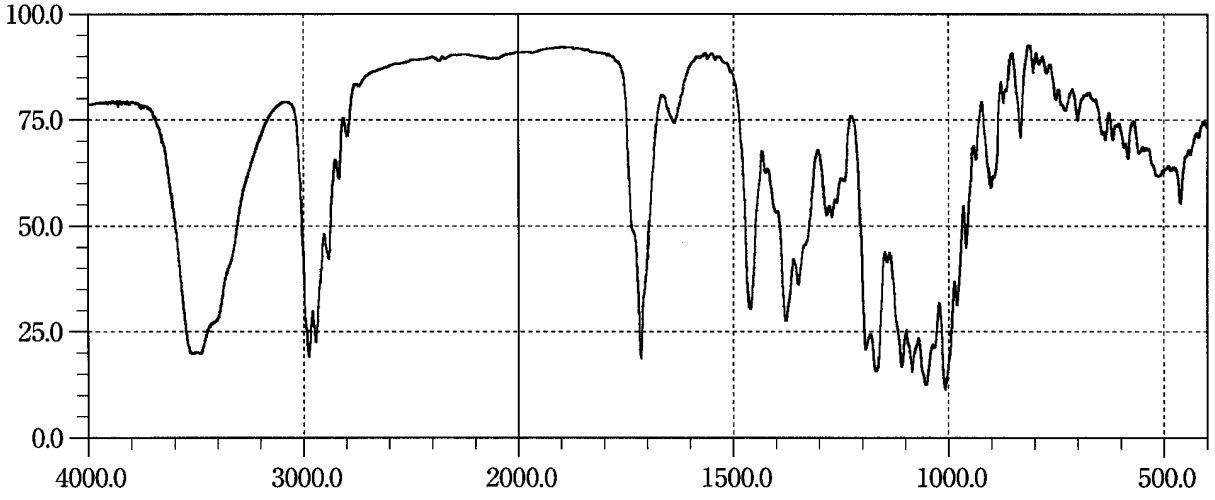
Preparation of sample: Potassium chloride disk method

Doxycycline Hydrochloride



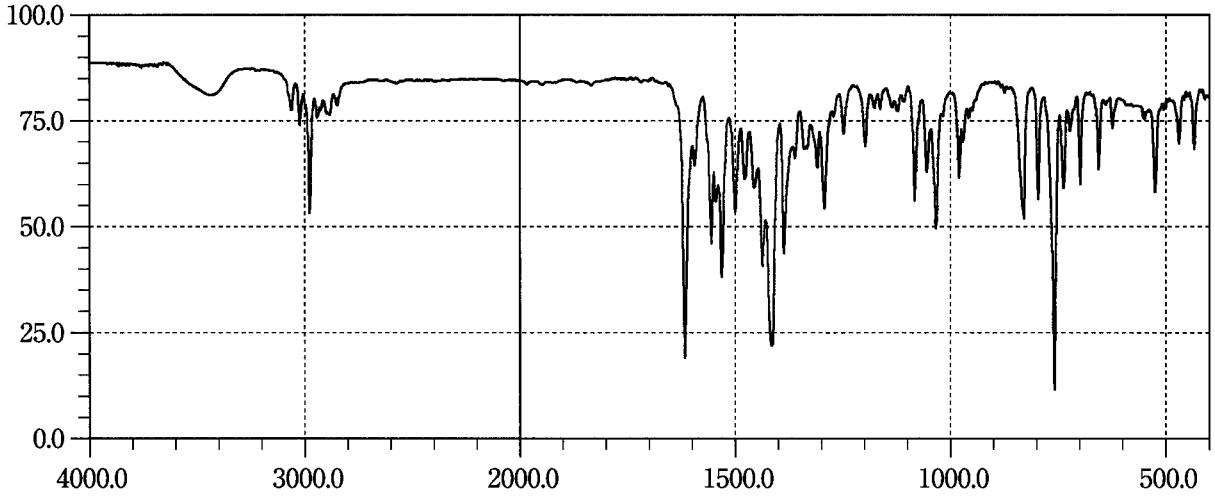
Preparation of sample: Potassium bromide disk method

Erythromycin



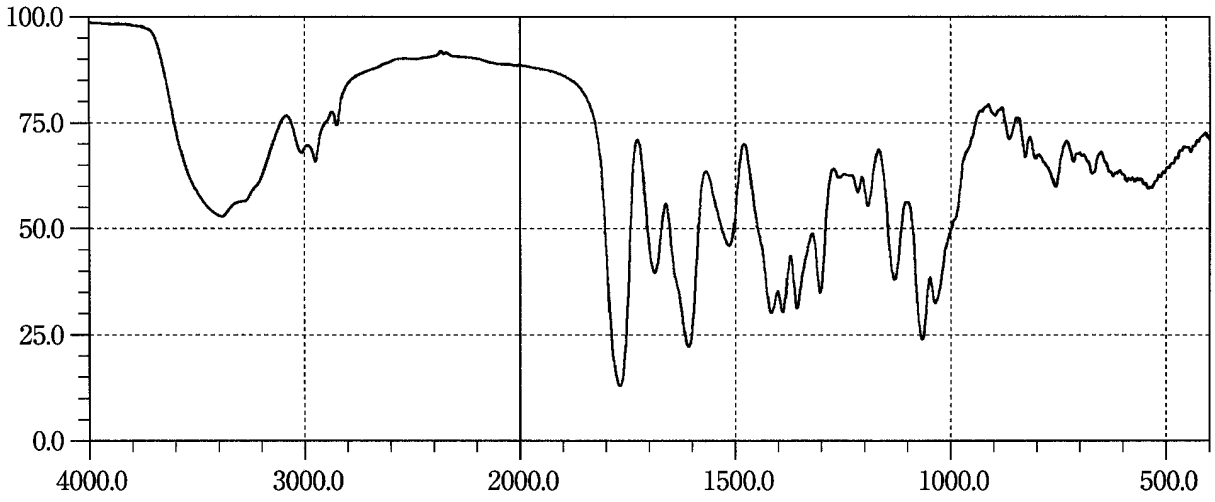
Preparation of sample: Potassium bromide disk method

Etizolam



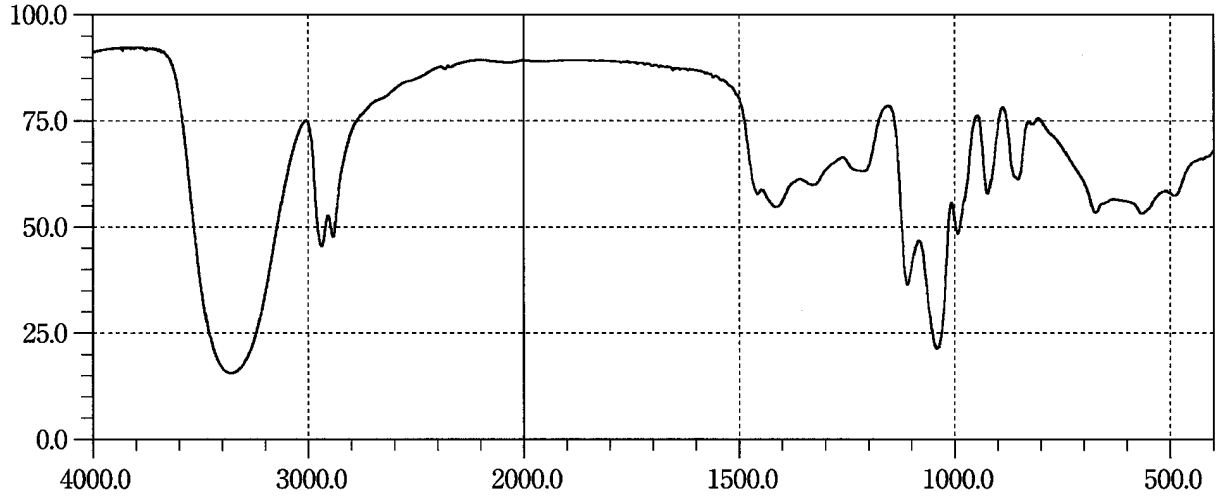
Preparation of sample: Potassium bromide disk method

Flomoxef Sodium



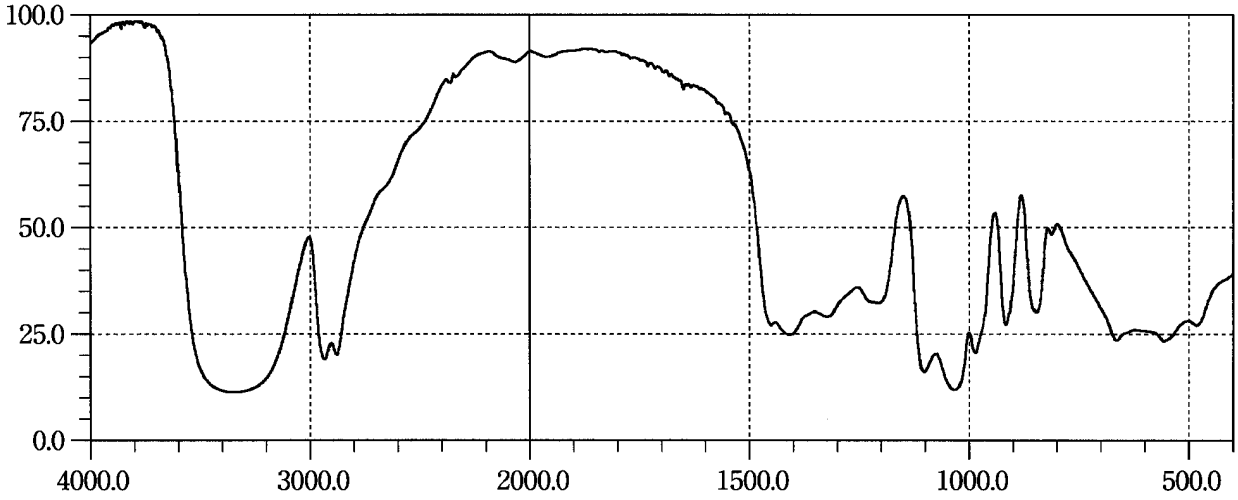
Preparation of sample: Potassium bromide disk method

Glycerin



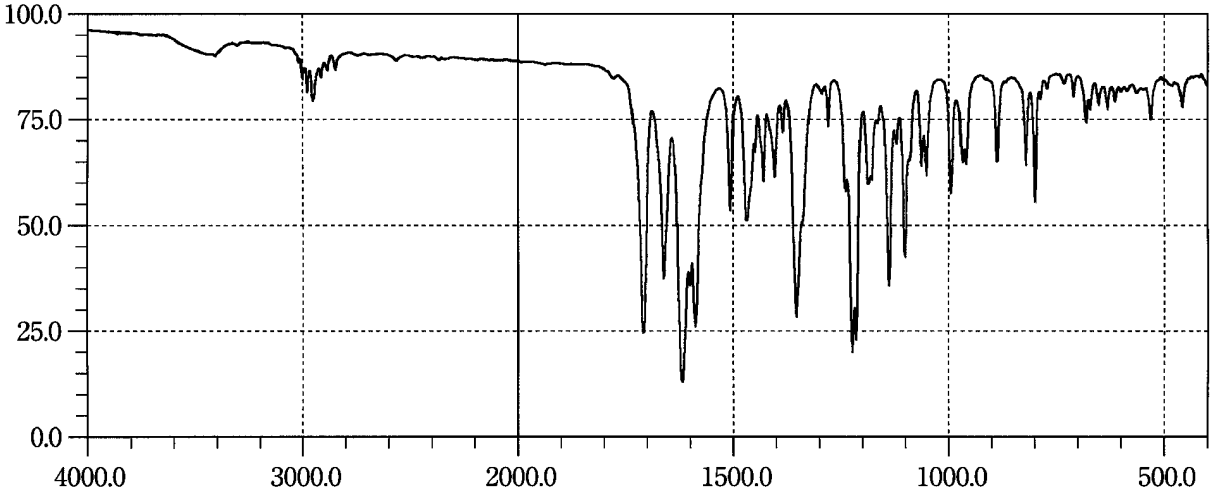
Preparation of sample: Liquid film method

Concentrated Glycerin



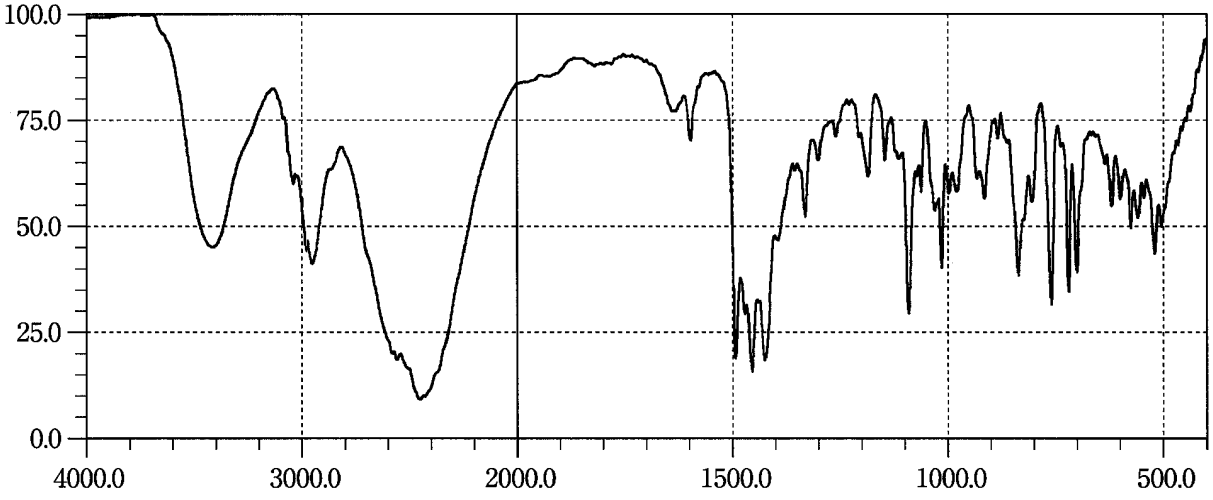
Preparation of sample: Liquid film method

Griseofulvin



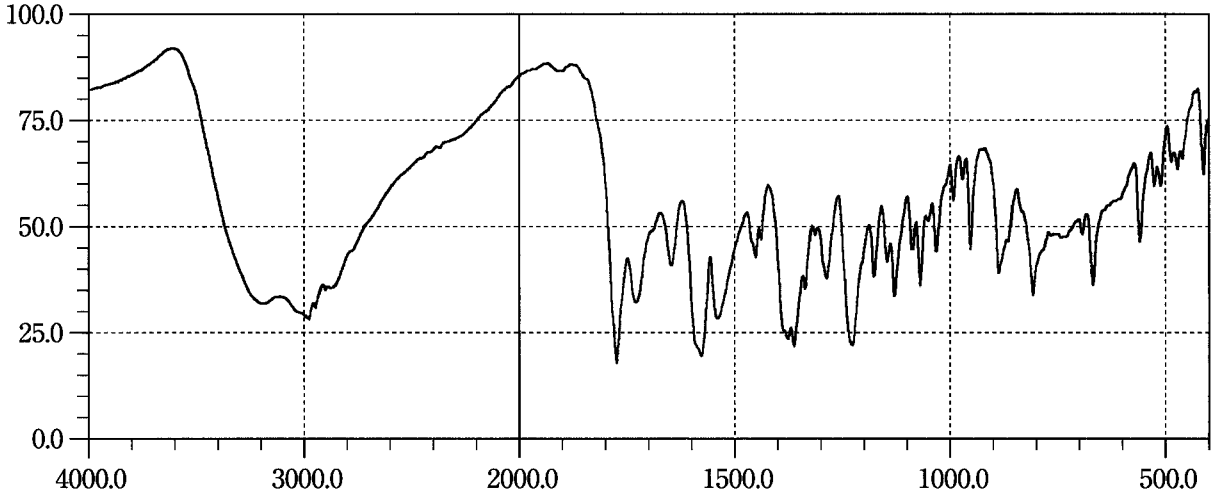
Preparation of sample: Potassium bromide disk method

Homochlorcyclizine Hydrochloride



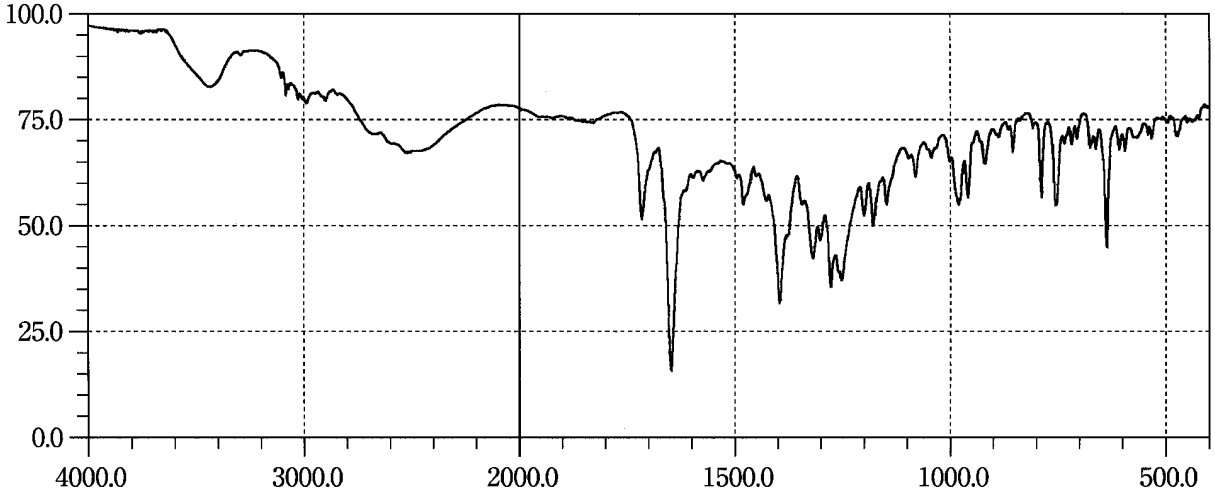
Preparation of sample: Potassium chloride disk method

Imipenem



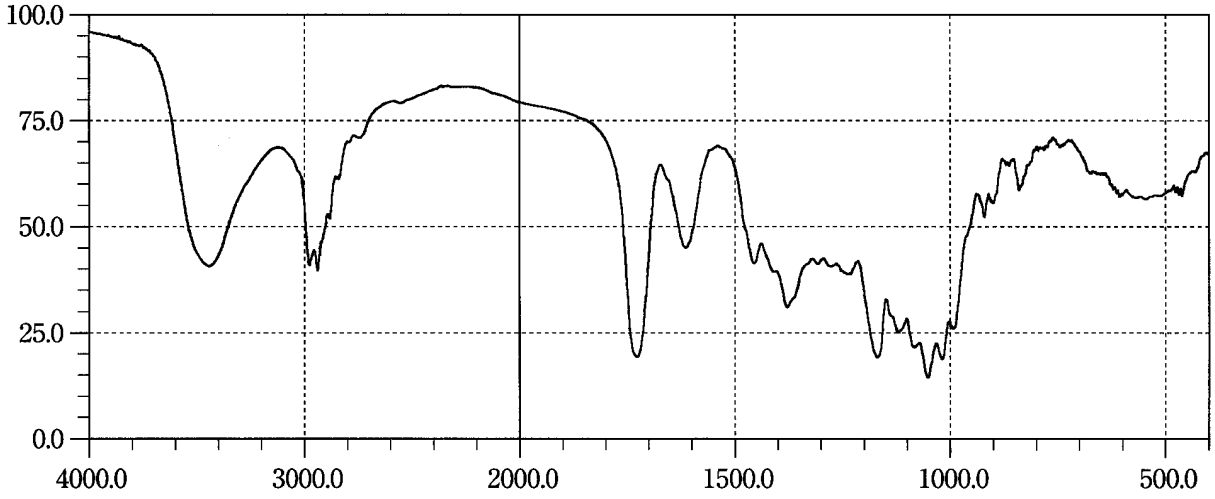
Preparation of sample: Potassium bromide disk method

Ketotifen Fumarate



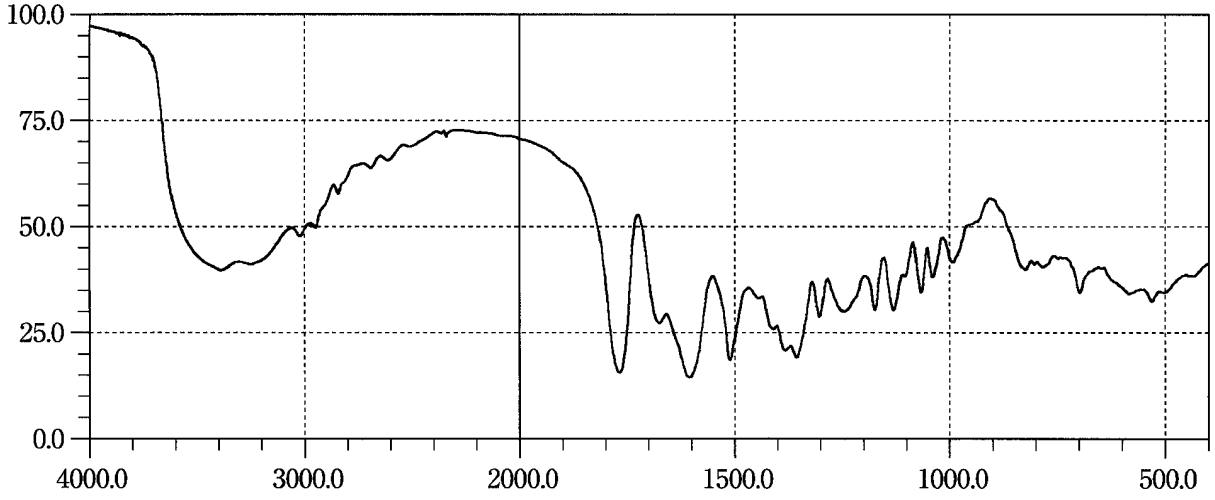
Preparation of sample: Potassium bromide disk method

Kitasamycin Tartrate



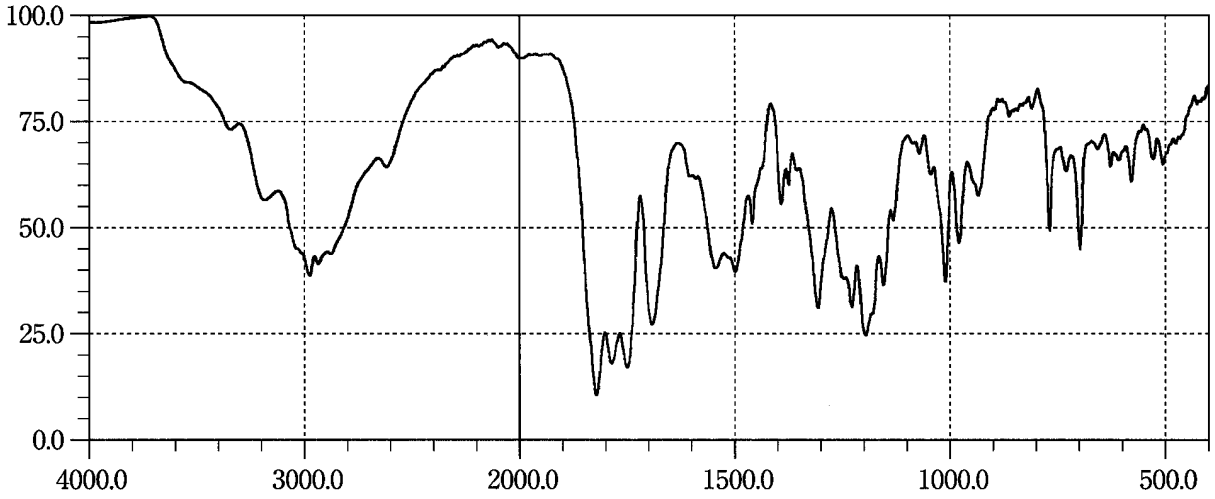
Preparation of sample: Potassium bromide disk method

Latamoxef Sodium



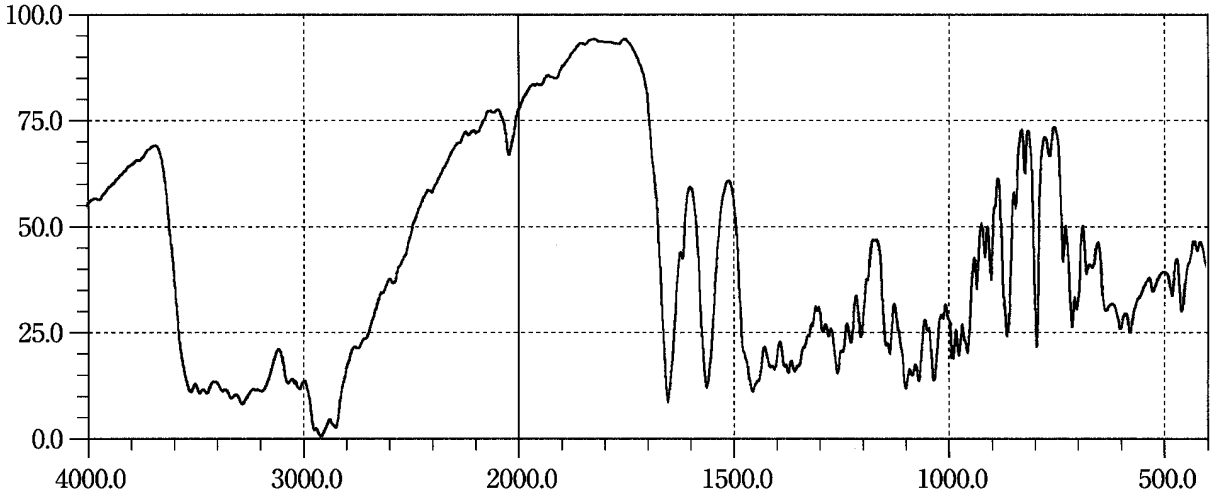
Preparation of sample: Potassium bromide disk method

Lenampicillin Hydrochloride



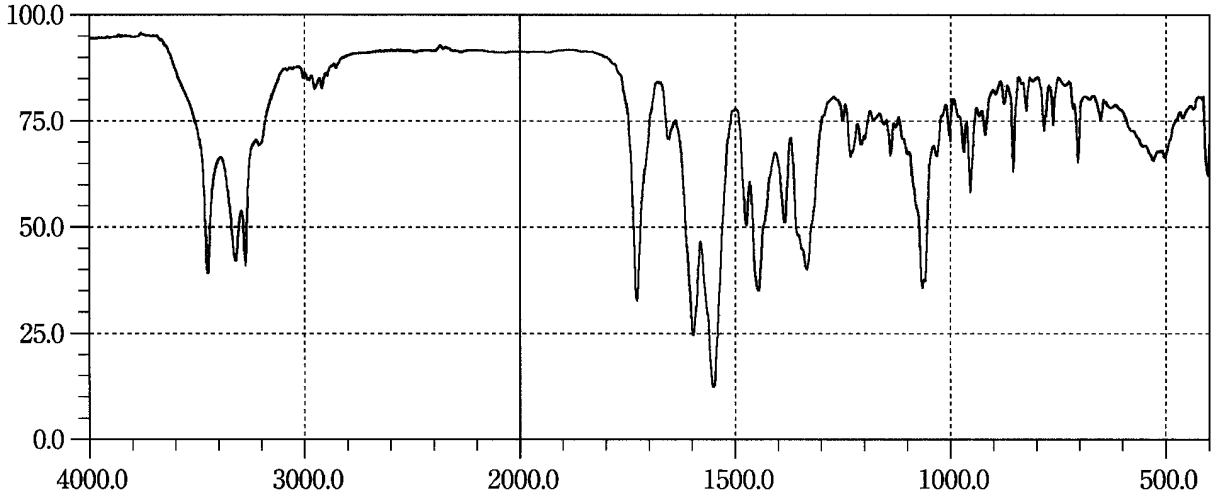
Preparation of sample: Potassium chloride disk method

Lincomycin Hydrochloride



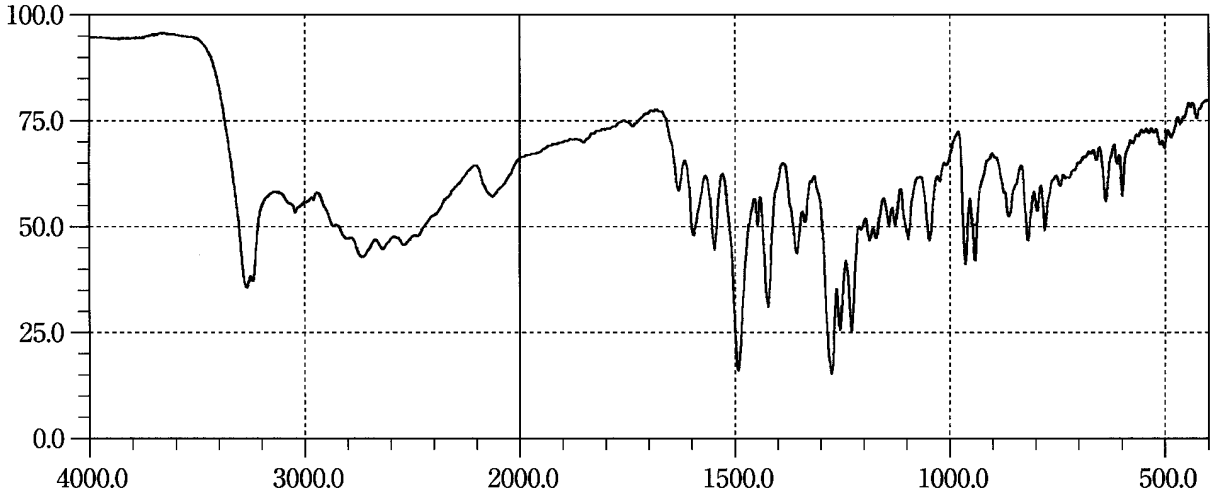
Preparation of sample: Paste method

Mitomycin C



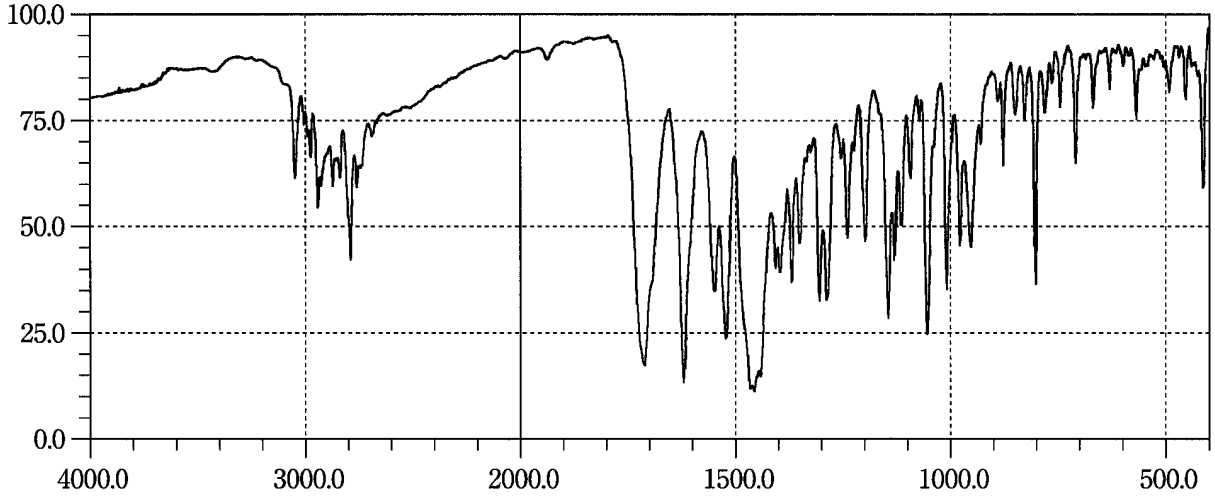
Preparation of sample: Potassium bromide disk method

Norepinephrine



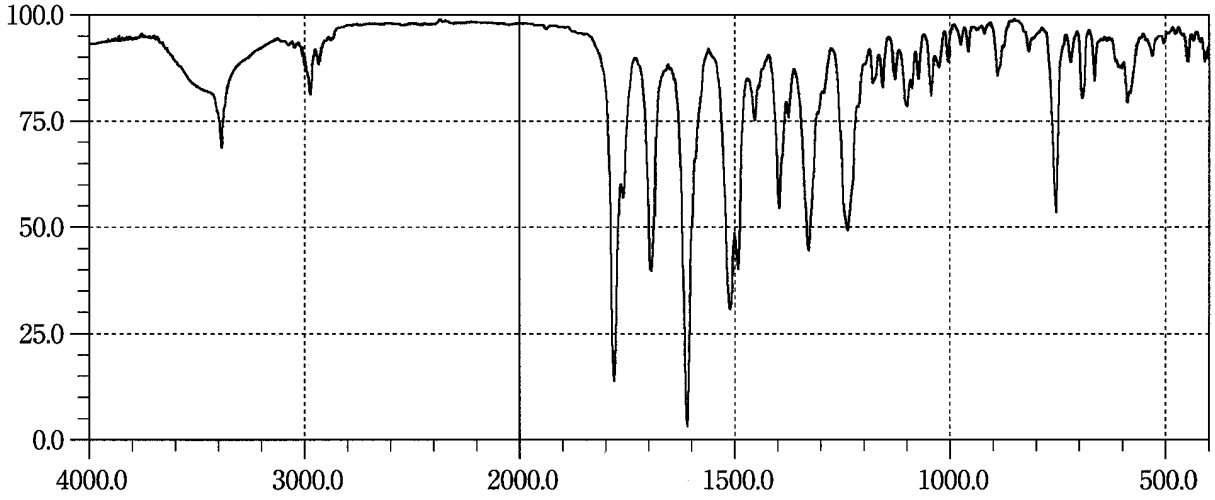
Preparation of sample: Potassium bromide disk method

Ofloxacin



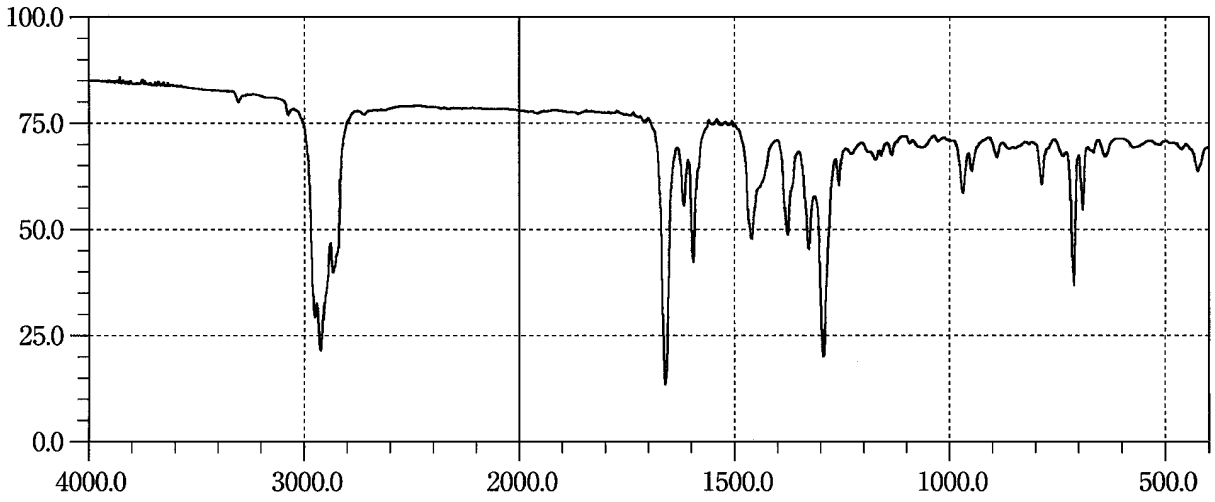
Preparation of sample: Potassium bromide disk method

Phenethicillin Potassium



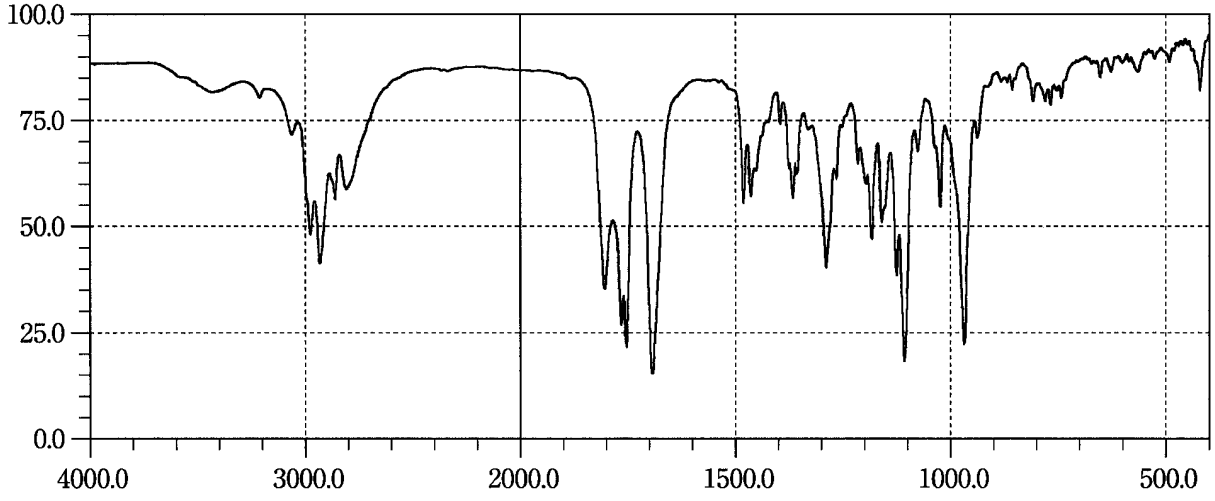
Preparation of sample: Potassium bromide disk method

Phytonadione



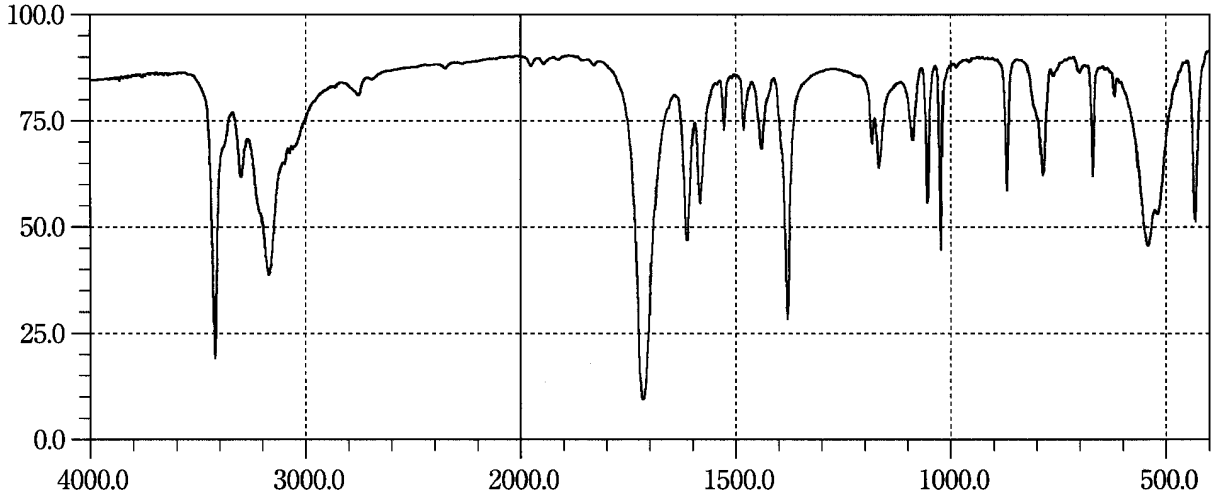
Preparation of sample: Liquid film method

Pivmecillinam Hydrochloride



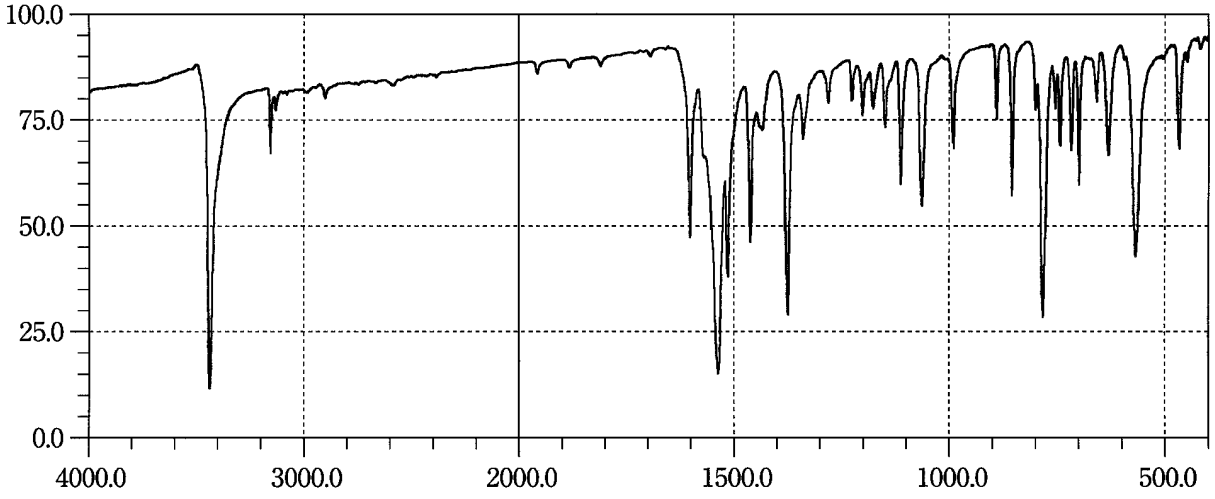
Preparation of sample: Potassium bromide disk method

Pyrazinamide



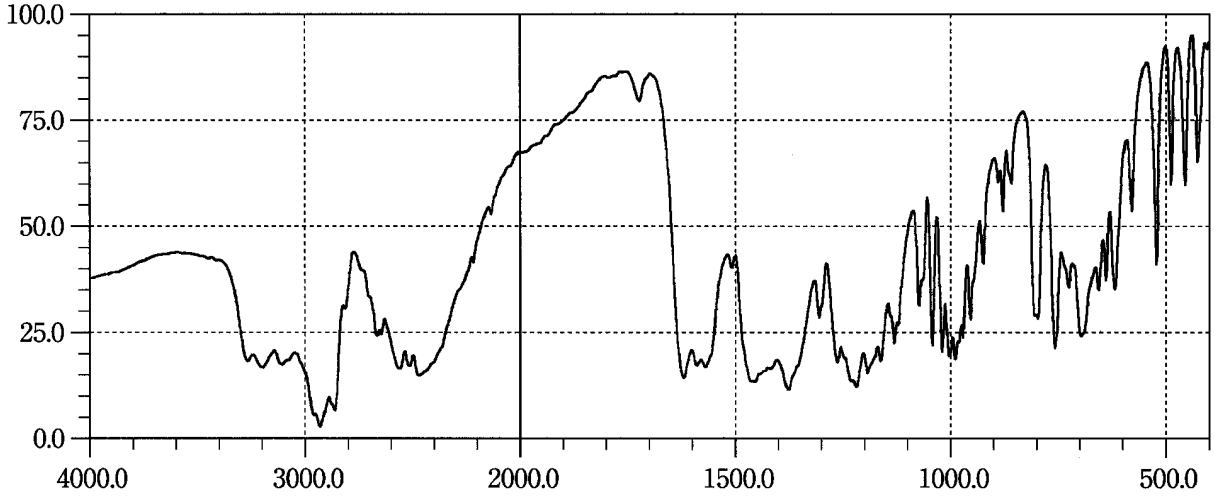
Preparation of sample: Potassium bromide disk method

Pyrrolnitrin



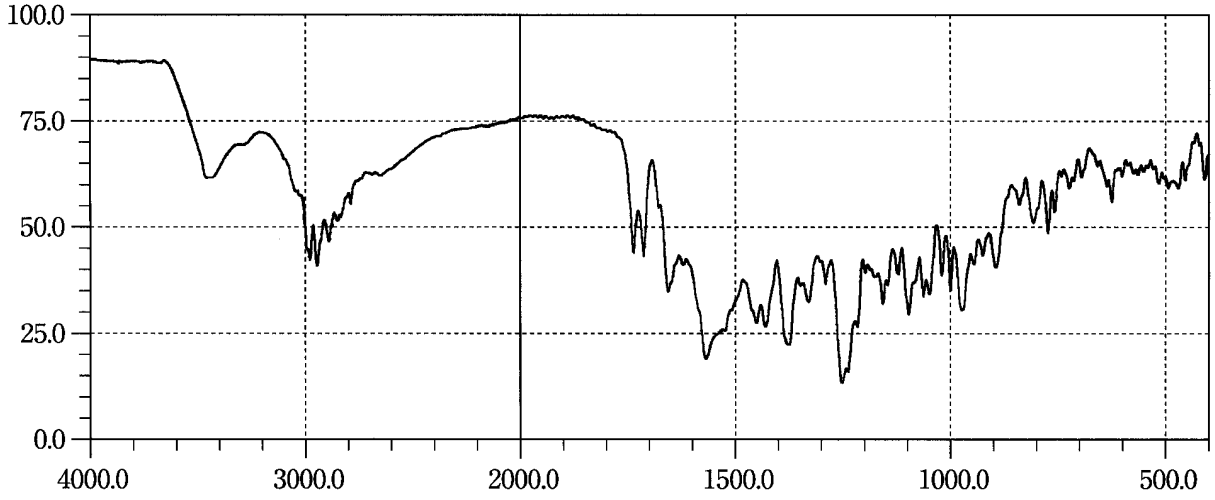
Preparation of sample: Potassium bromide disk method

Ranitidine Hydrochloride



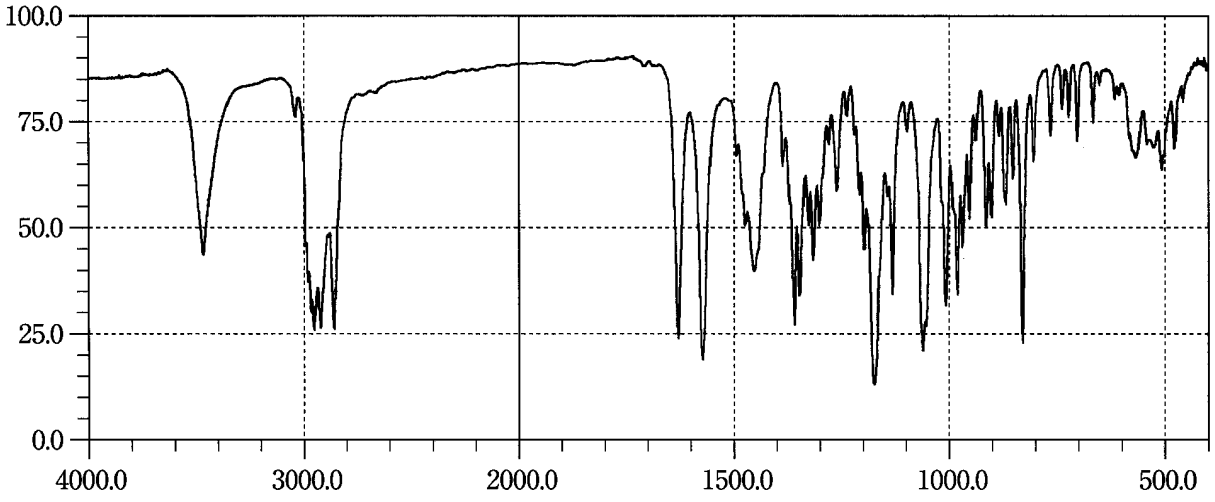
Preparation of sample: Paste method

Rifampicin



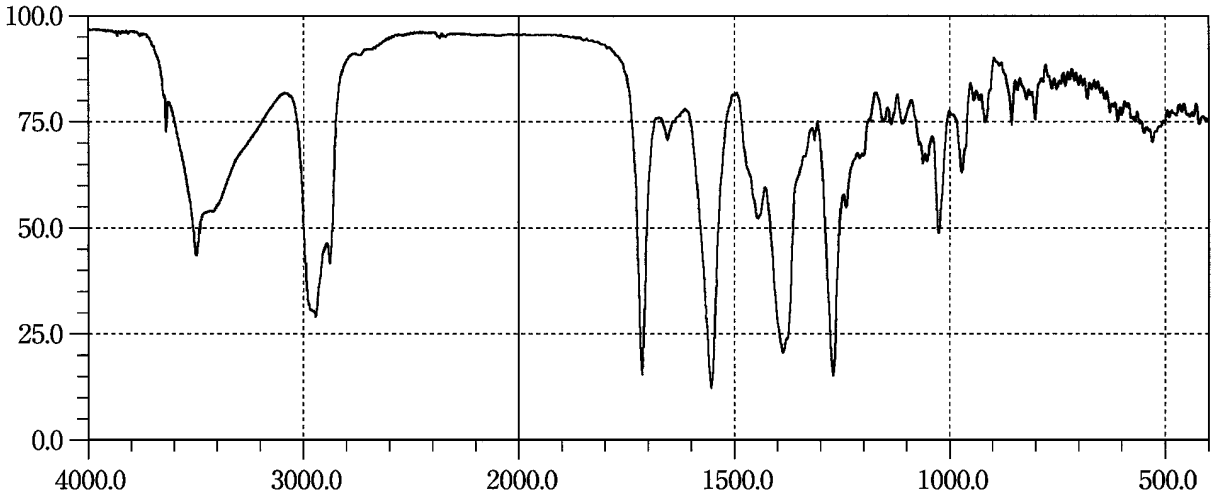
Preparation of sample: Potassium bromide disk method

Siccanin



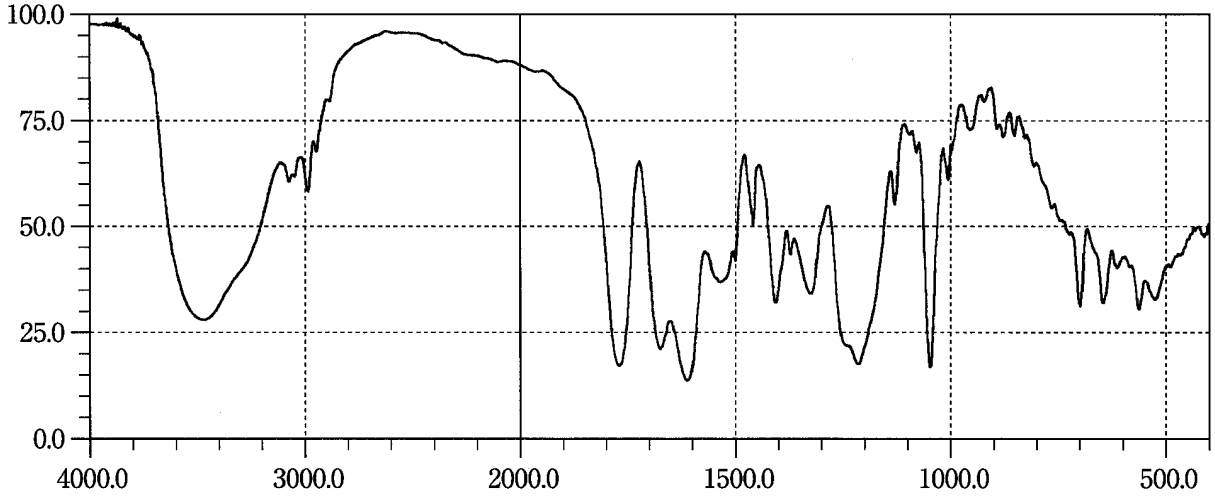
Preparation of sample: Potassium bromide disk method

Sodium Fusidate



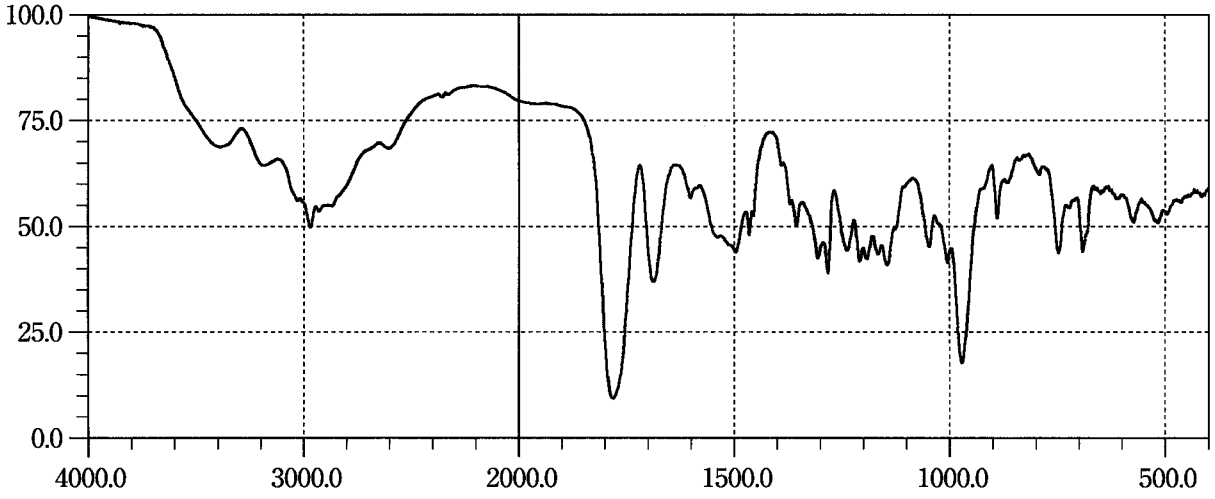
Preparation of sample: Potassium bromide disk method

Sulbenicillin Sodium



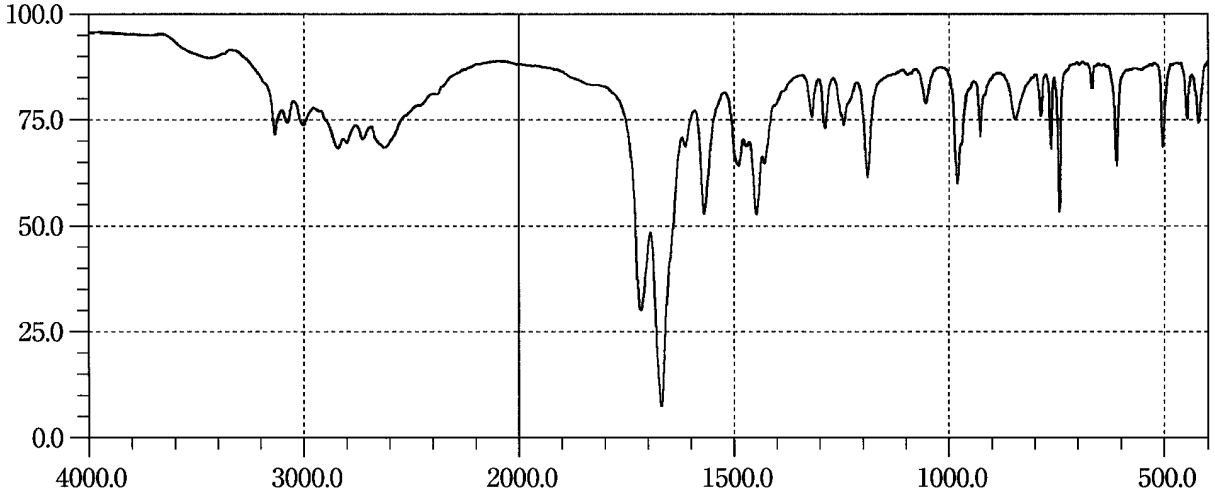
Preparation of sample: Potassium bromide disk method

Talampicillin Hydrochloride



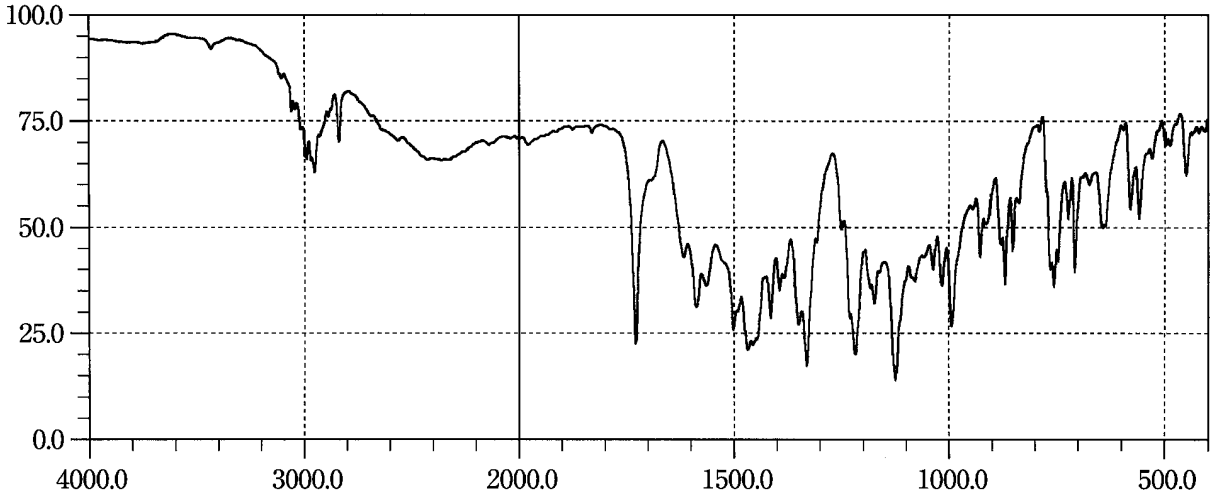
Preparation of sample: Potassium bromide disk method

Theophylline



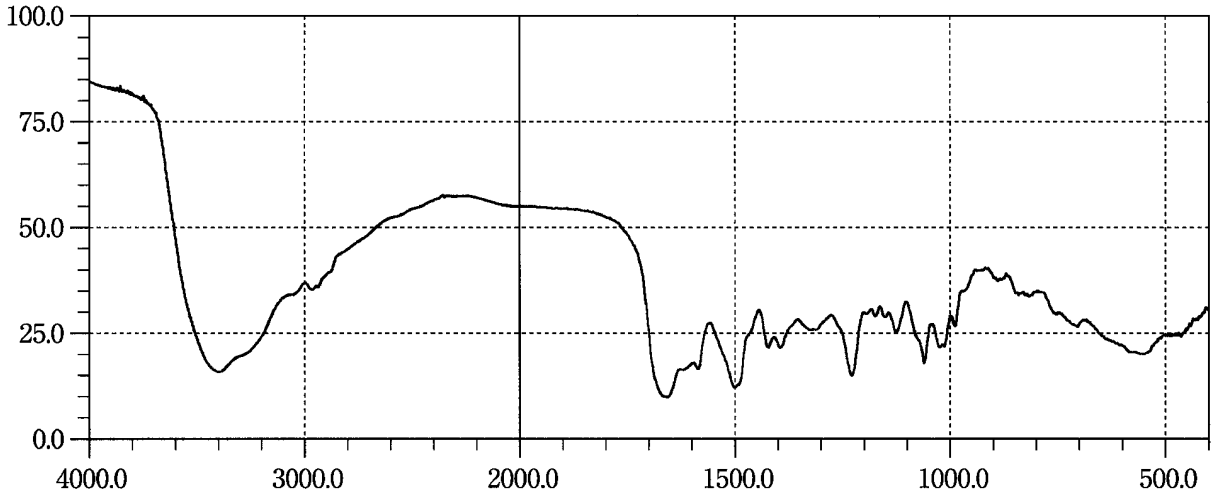
Preparation of sample: Potassium bromide disk method

Trimebutine Maleate



Preparation of sample: Potassium bromide disk method

Vancomycin Hydrochloride



Preparation of sample: Potassium bromide disk method

Delete the following Ultraviolet-visible Reference Spectra:

Part I

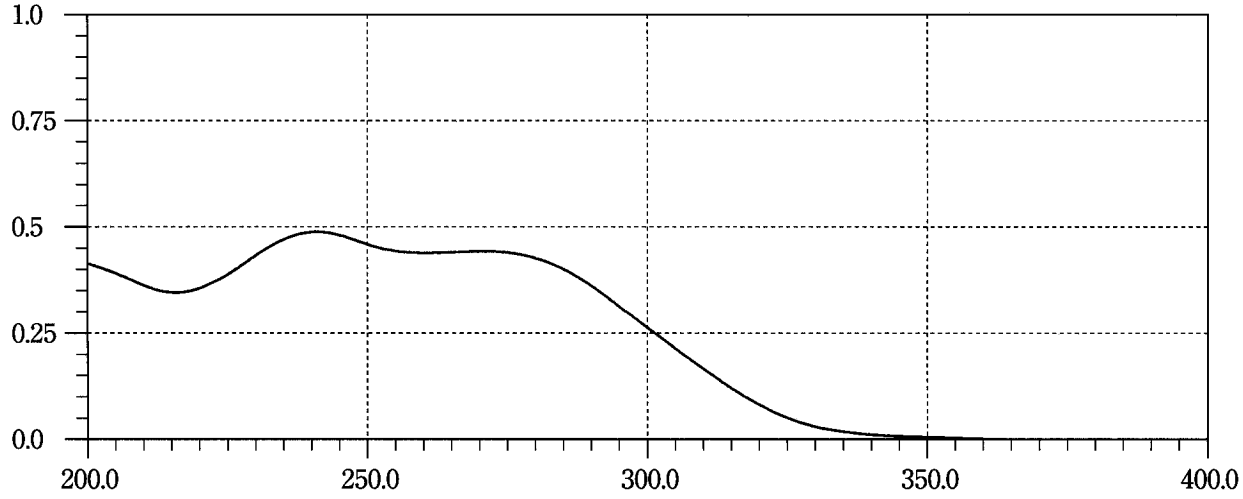
Homochlorcyclizine Hydrochloride 1

Homochlorcyclizine Hydrochloride 2

Change to read:

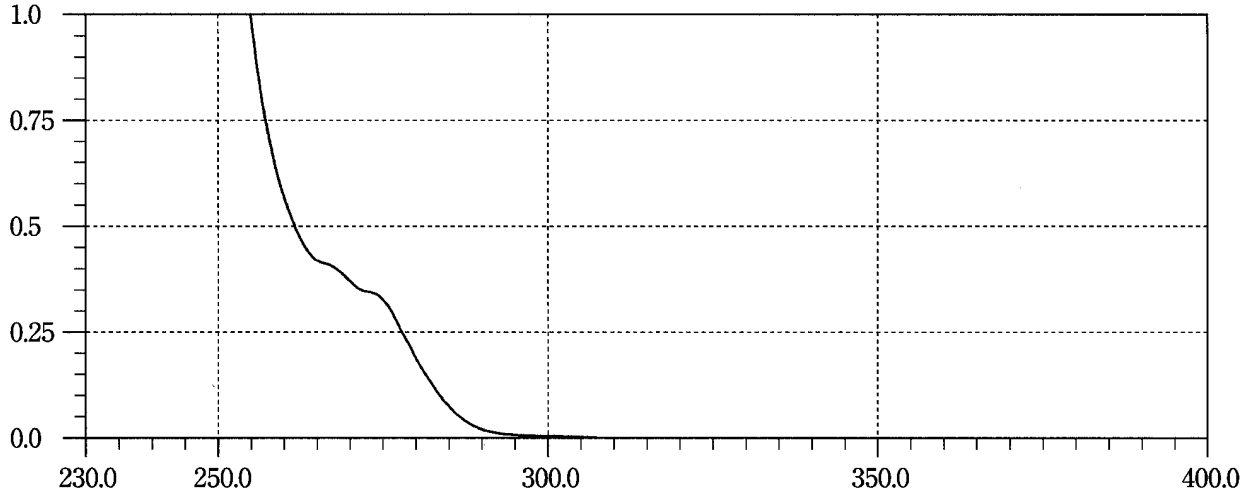
Part I

Ceftriaxone Sodium



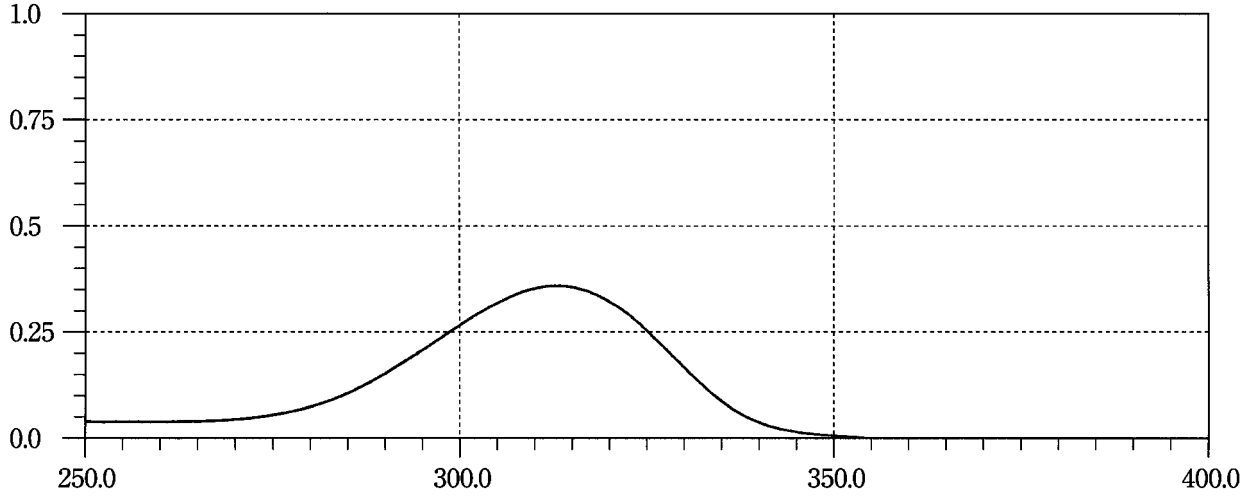
A solution of Ceftriaxone Sodium (1 in 100,000)

Cloxacillin Sodium



A solution of Cloxacillin Sodium in methanol (1 in 2500)

Potassium Clavulanate

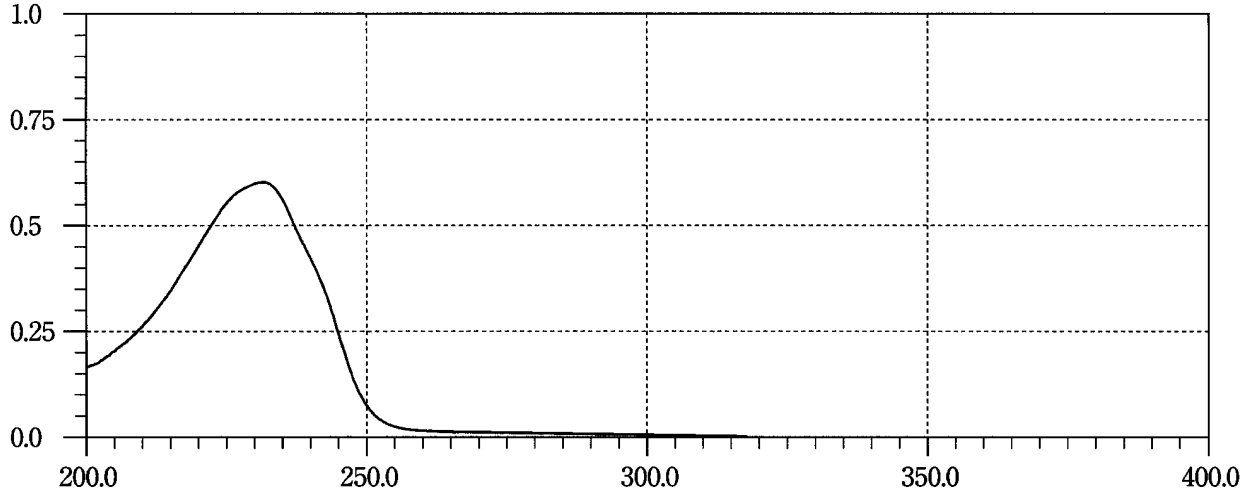


A solution prepared as follows: To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30℃ for 12 minutes.

Add the following:

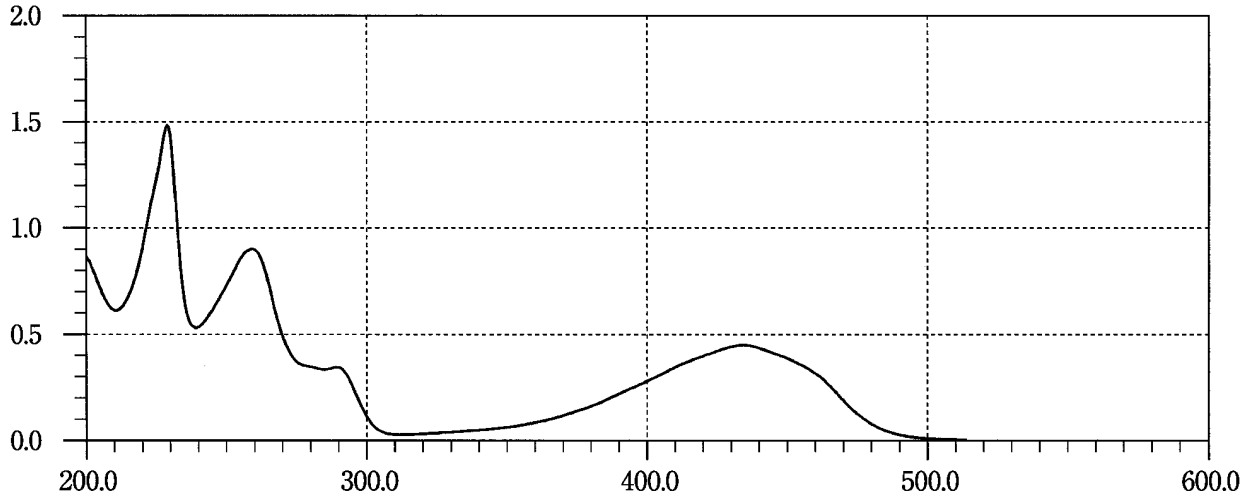
Part I

Acetylspiramycin



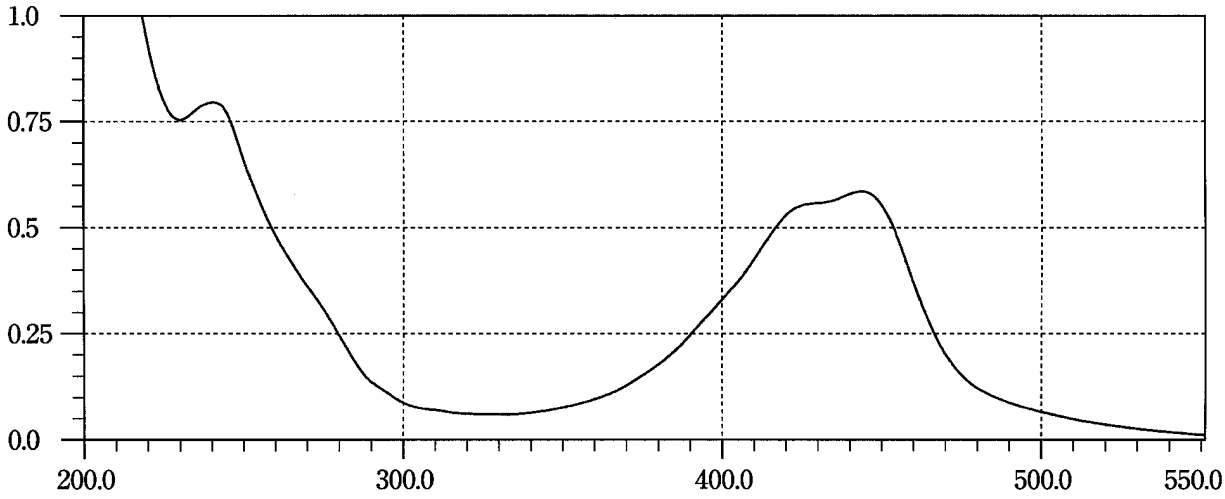
A solution of Acetylspiramycin in methanol (1 in 50,000)

Aclarubicin Hydrochloride



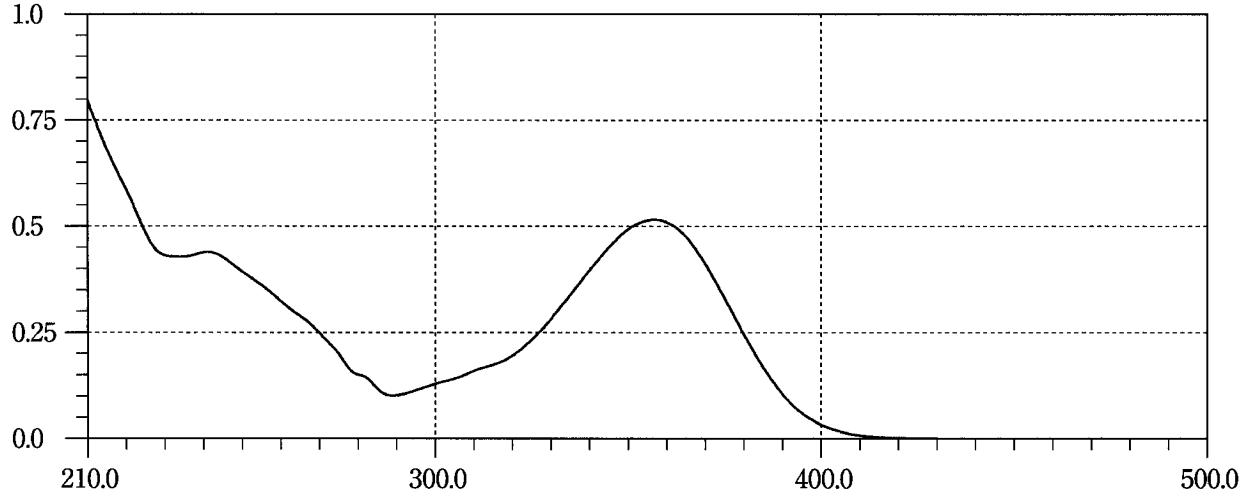
A solution of Aclarubicin Hydrochloride in diluted methanol (4 in 5) (3 in 100,000)

Actinomycin D



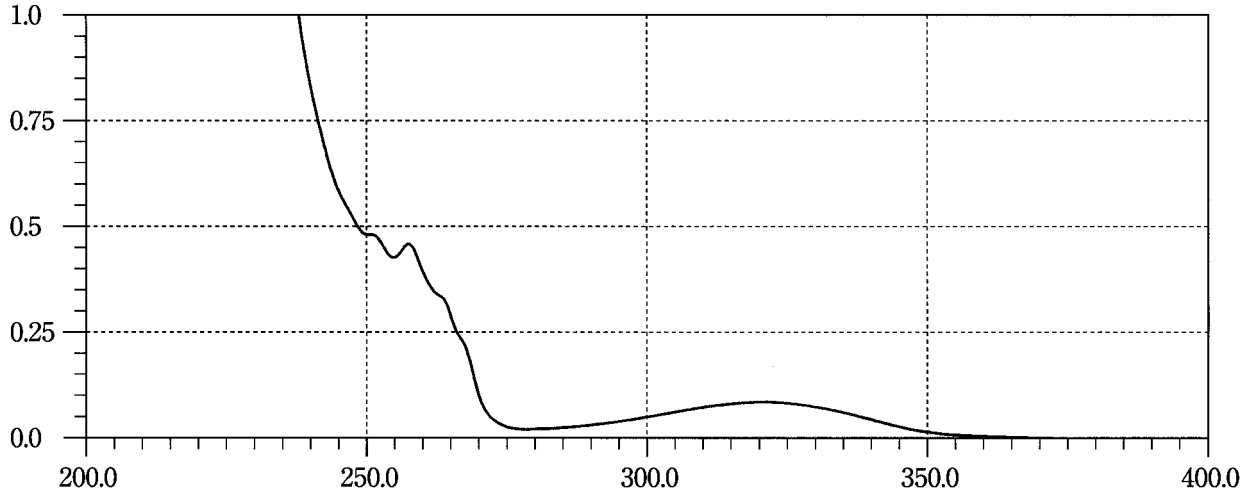
A solution of Actinomycin D in methanol (3 in 100,000)

Benzbromarone



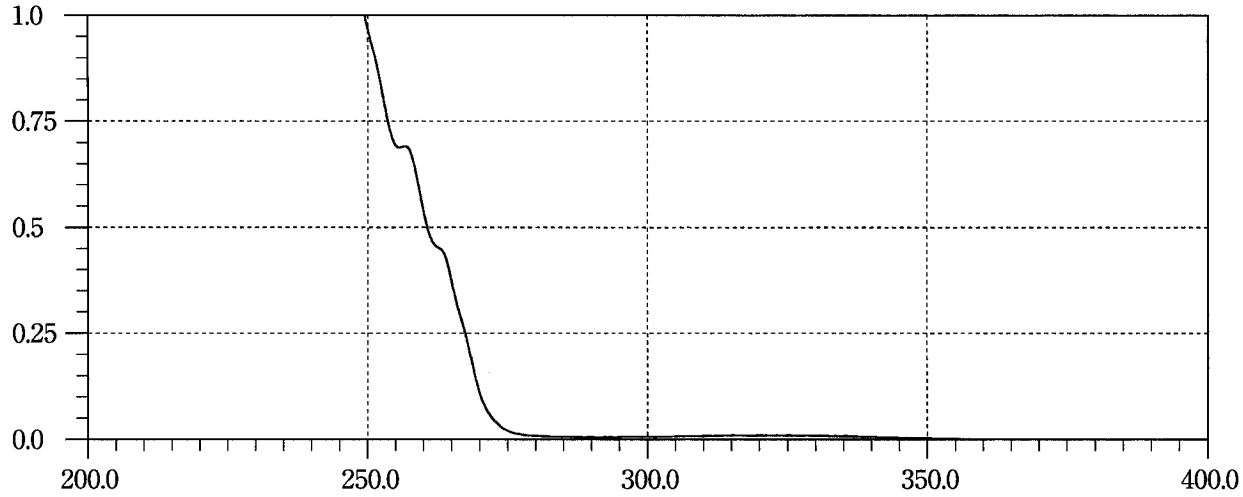
A solution of Benzbromarone in 0.01 mol/L sodium hydroxide TS (1 in 100,000)

Benzylpenicillin Benzathine



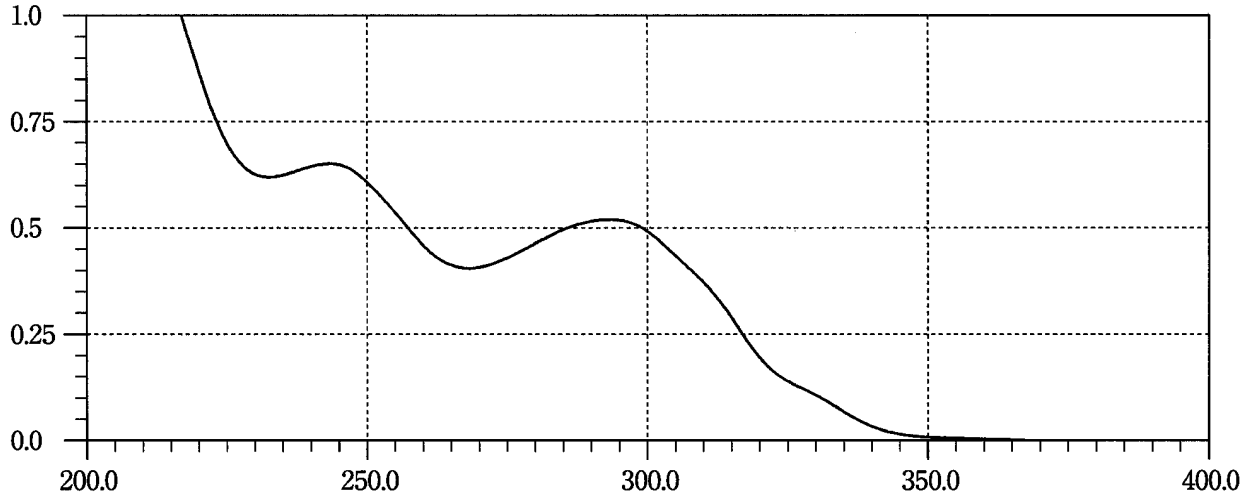
A solution of Benzylpenicillin Benzathine in methanol (1 in 2000)

Benzylpenicillin Potassium



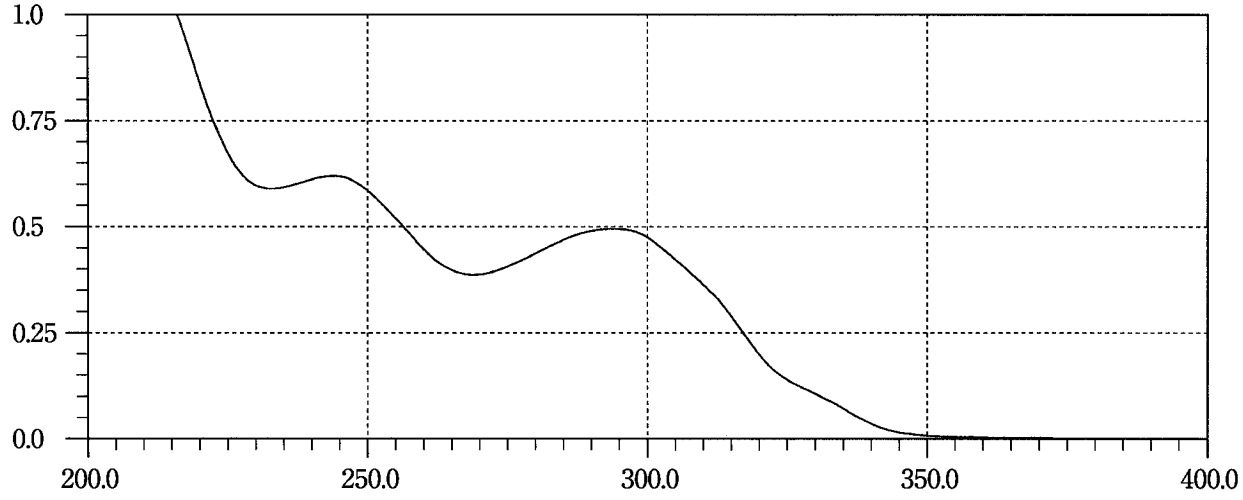
A solution of Benzylpenicillin Potassium (1 in 1000)

Bleomycin Hydrochloride



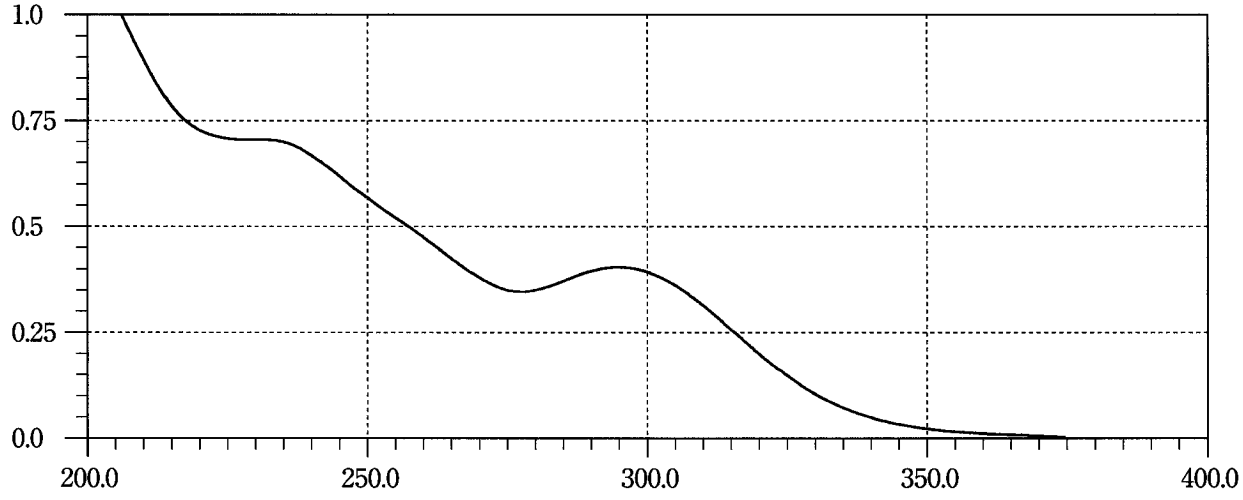
A solution prepared as follows: To 4 mg of Bleomycin Hydrochloride add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL.

Bleomycin Sulfate



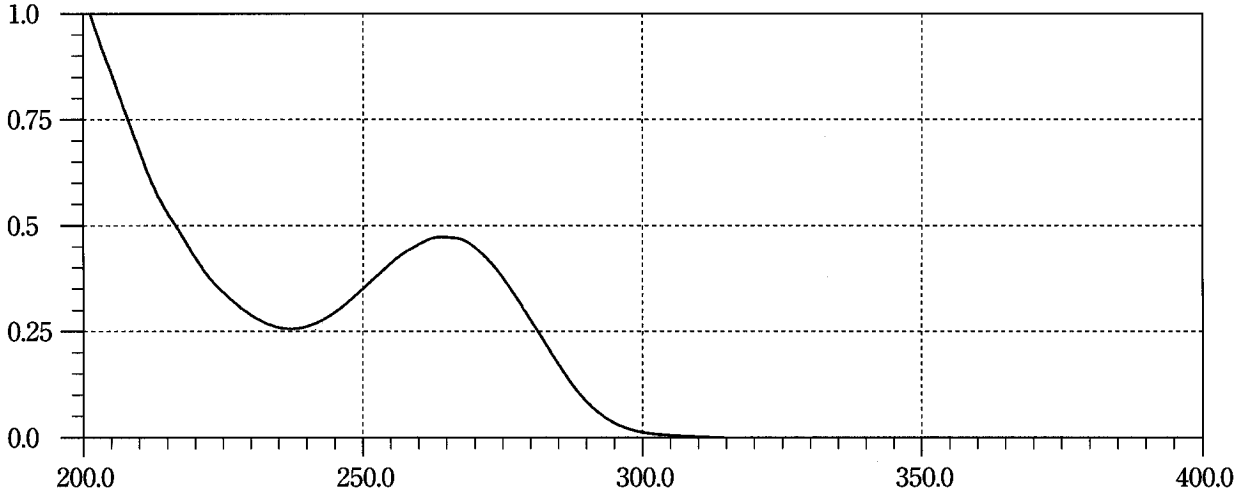
A solution prepared as follows: To 4 mg of Bleomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL.

Carumonam Sodium



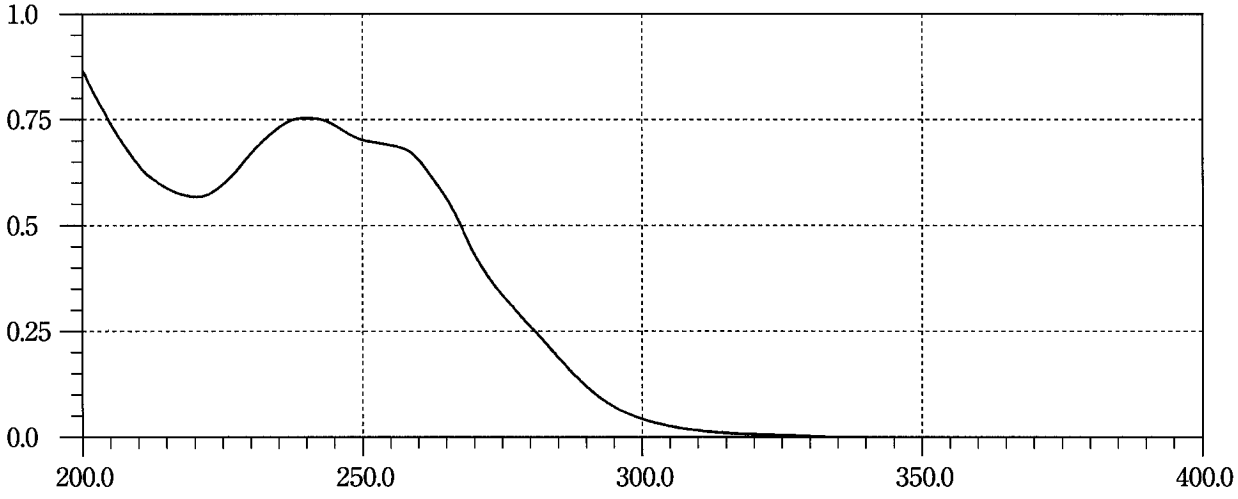
A solution of Carumonam Sodium (3 in 100,000)

Cefaclor



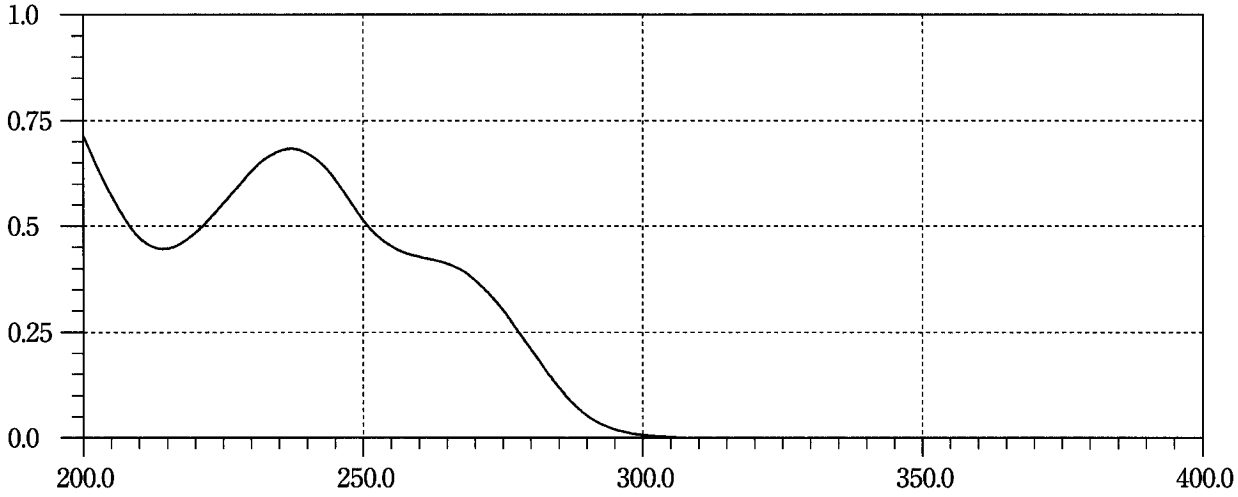
A solution of Cefaclor (1 in 50,000)

Cefaloridin



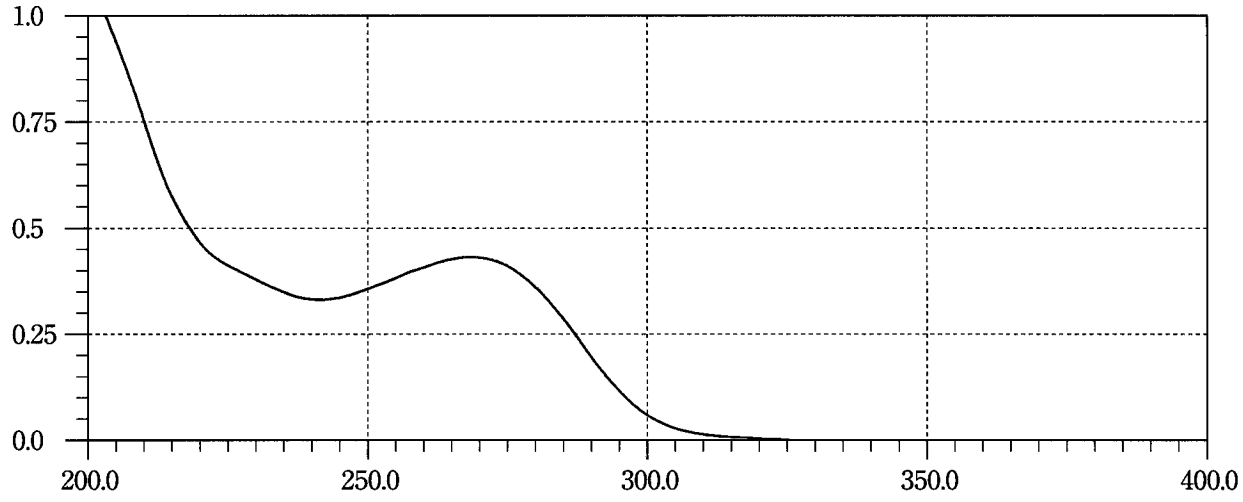
A solution of Cefaloridin (1 in 50,000)

Cefalotin Sodium



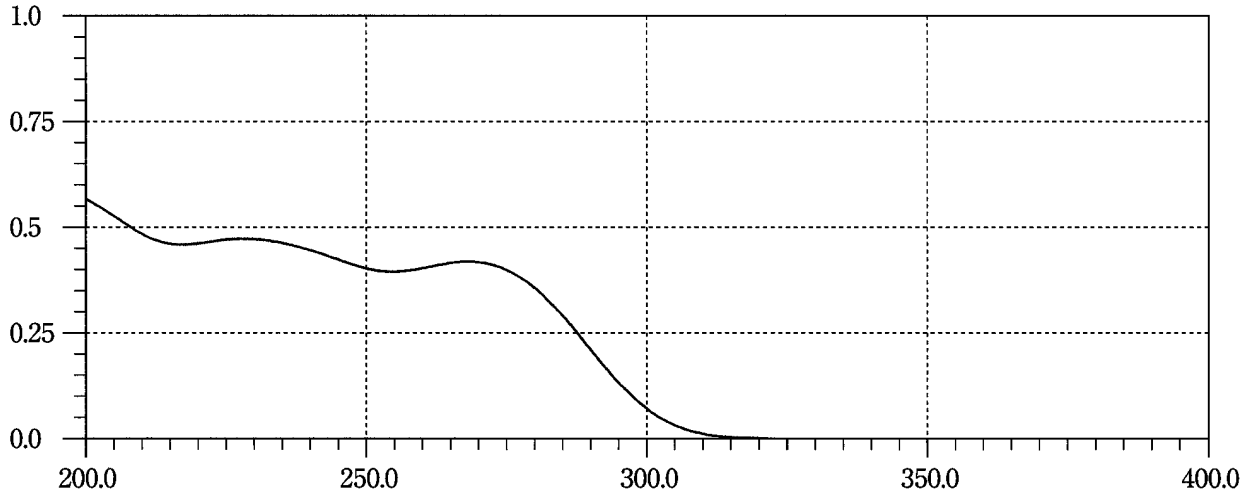
A solution of Cefalotin Sodium (1 in 50,000)

Cefamandole Sodium



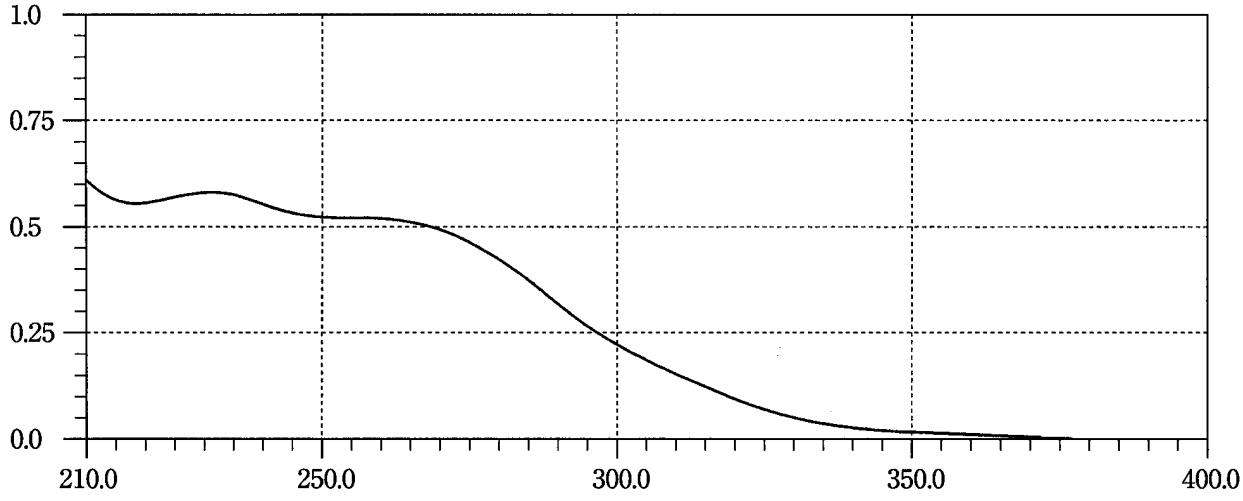
A solution of Cefamandole Sodium (1 in 50,000)

Cefbuperazone Sodium



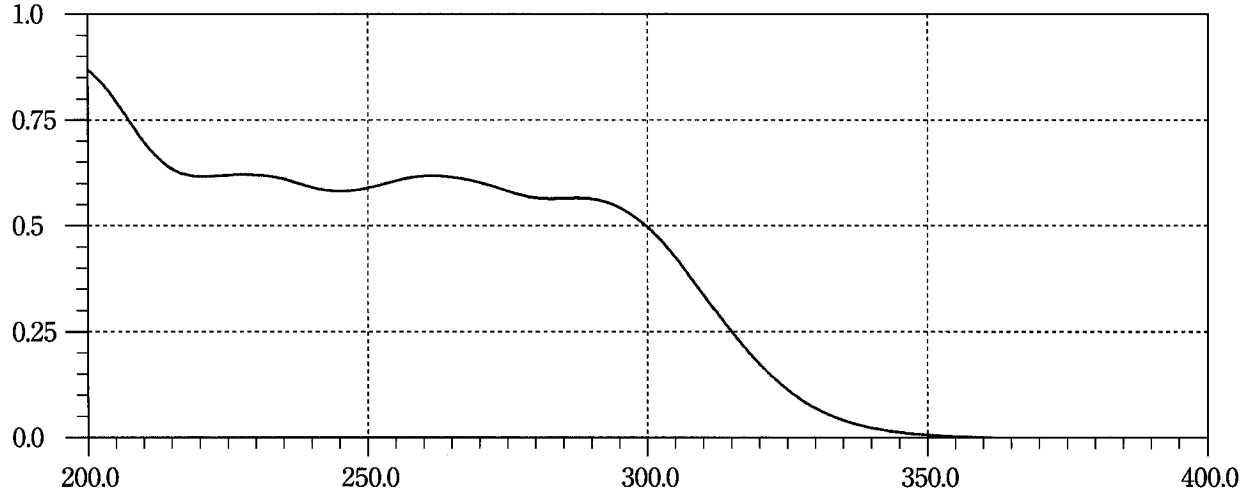
A solution of Cefbuperazone Sodium (1 in 50,000)

Cefmenoxime Hydrochloride



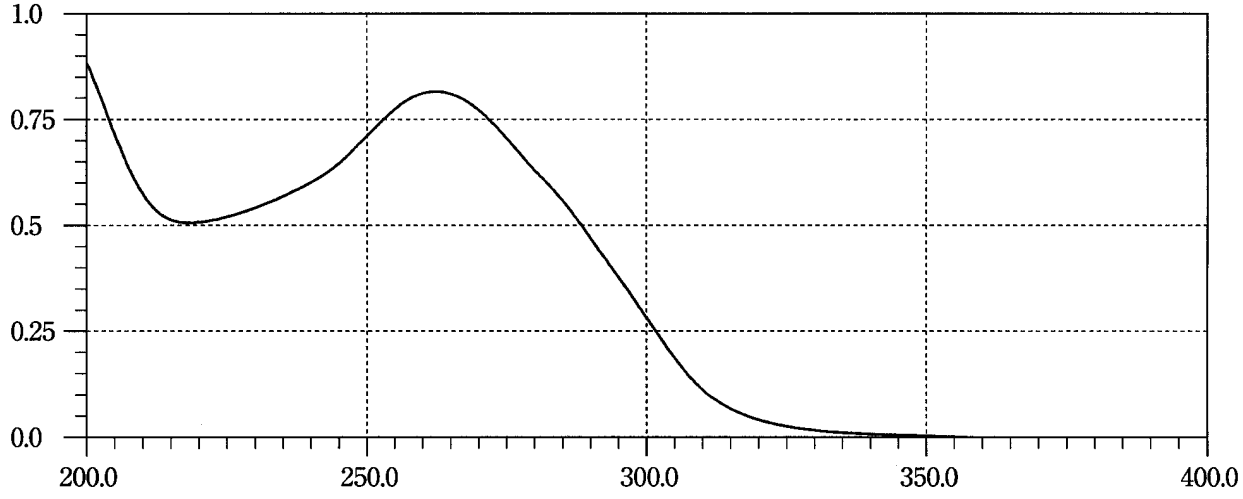
A solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution, pH 6.8 (3 in 200,000)

Cefodizime Sodium



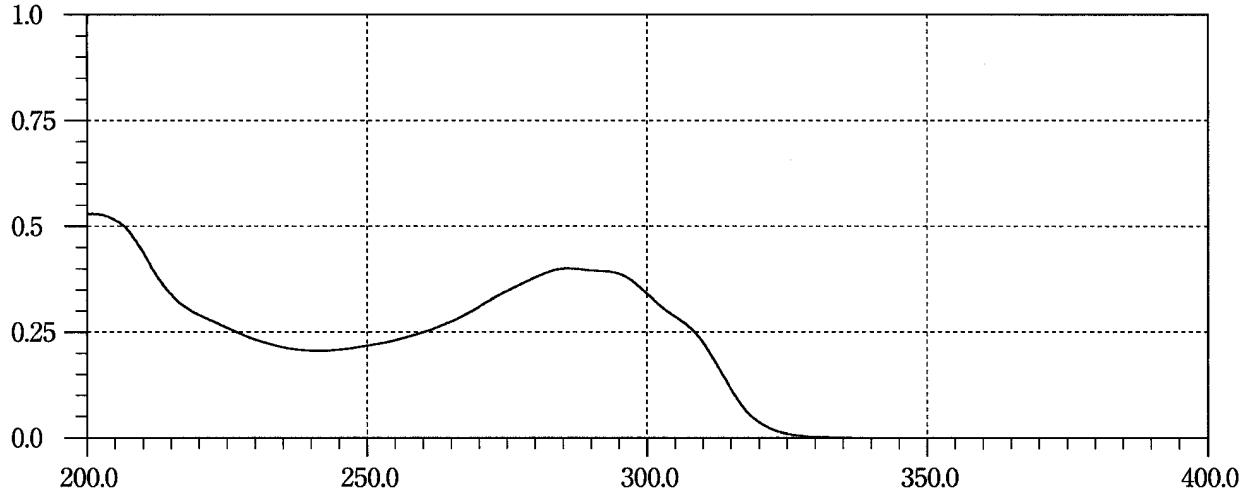
A solution of Cefodizime Sodium (1 in 50,000)

Cefotaxime Sodium



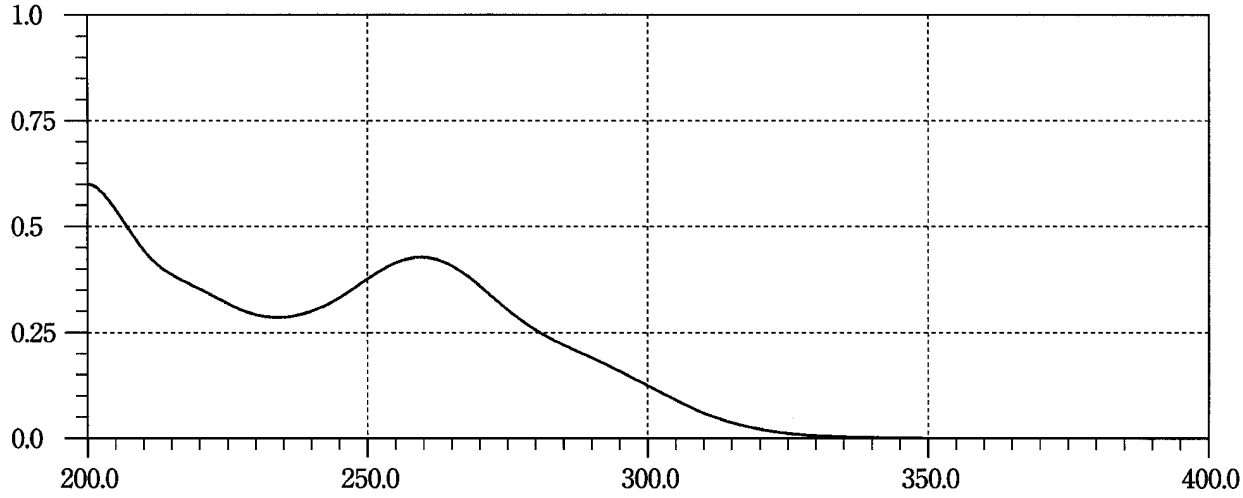
A solution of Cefotaxime Sodium in 0.01 mol/L hydrochloric acid TS (1 in 50,000)

Cefotetan



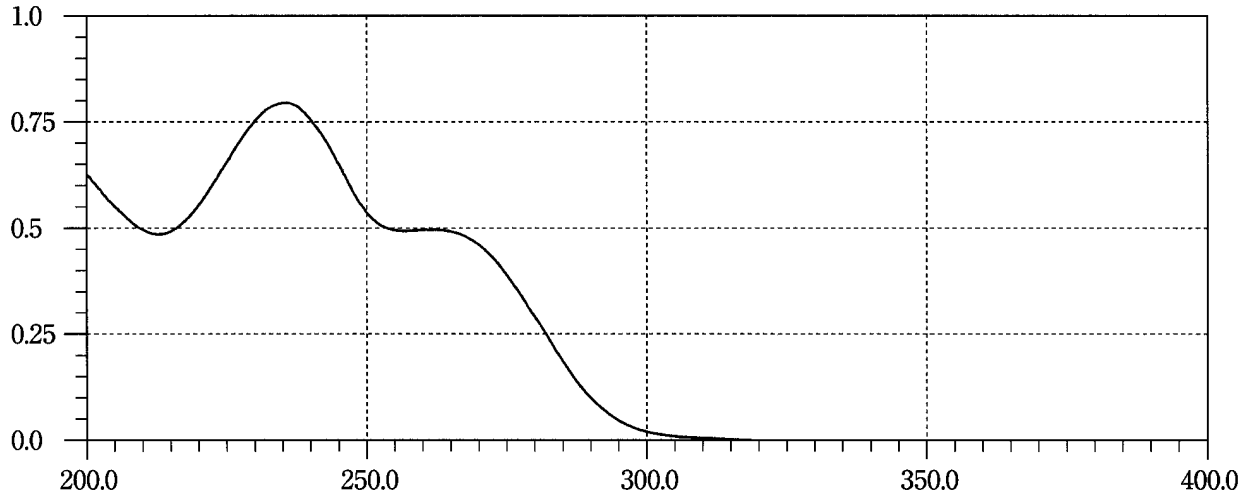
A solution of Cefotetan in phosphate buffer solution for antibiotics, pH 6.5 (1 in 100,000)

Cefotiam Hexetil Hydrochloride



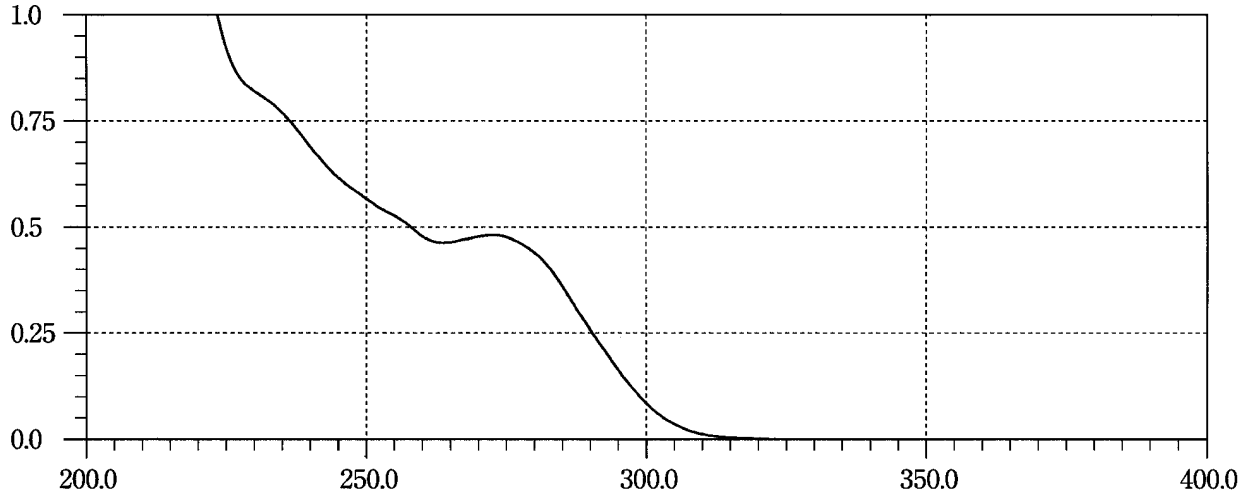
A solution of Cefotiam Hexetil Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 125,000)

Cefoxitin Sodium



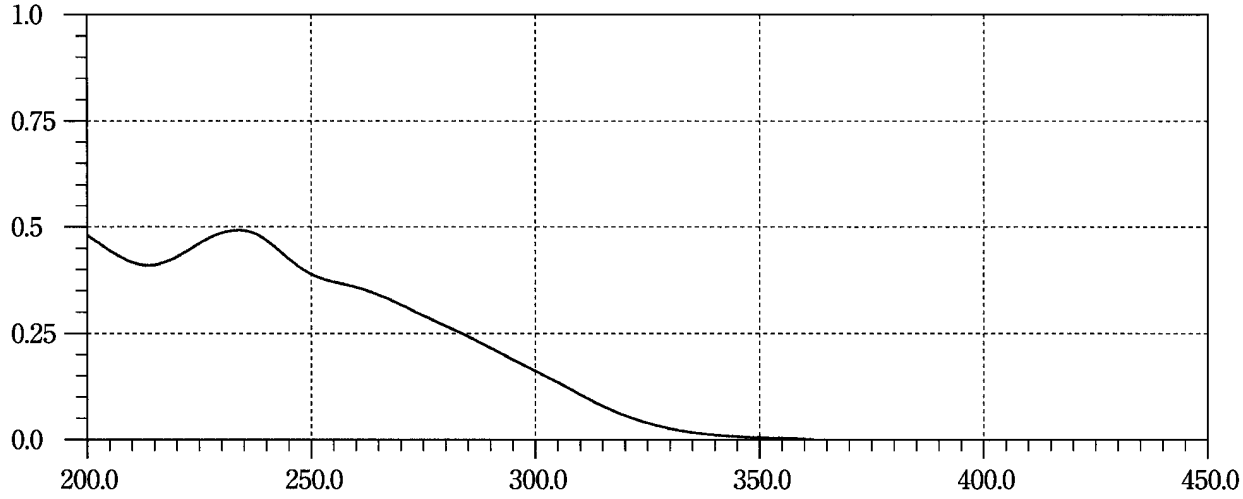
A solution of Cefoxitin Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 40,000)

Cefpiramide Sodium



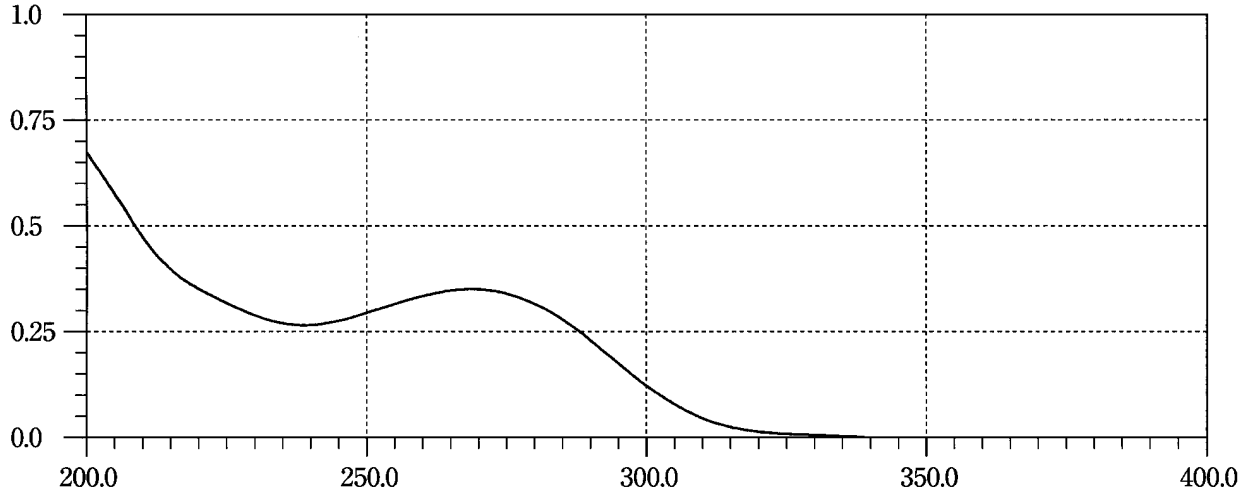
A solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 50,000)

Cefpodoxime Proxetil



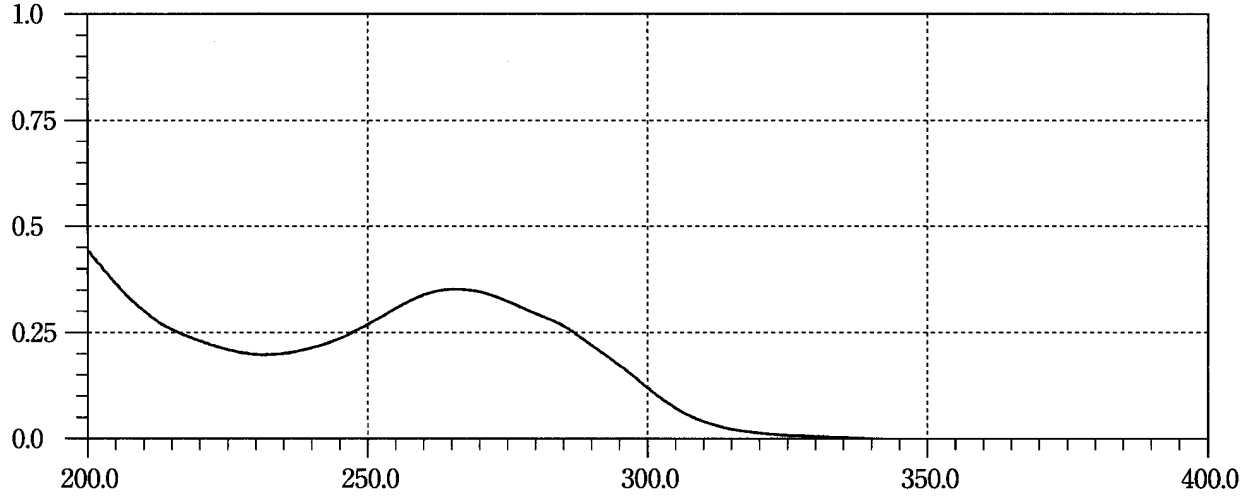
A solution of Cefpodoxime Proxetil in acetonitrile (3 in 200,000)

Cefroxadine



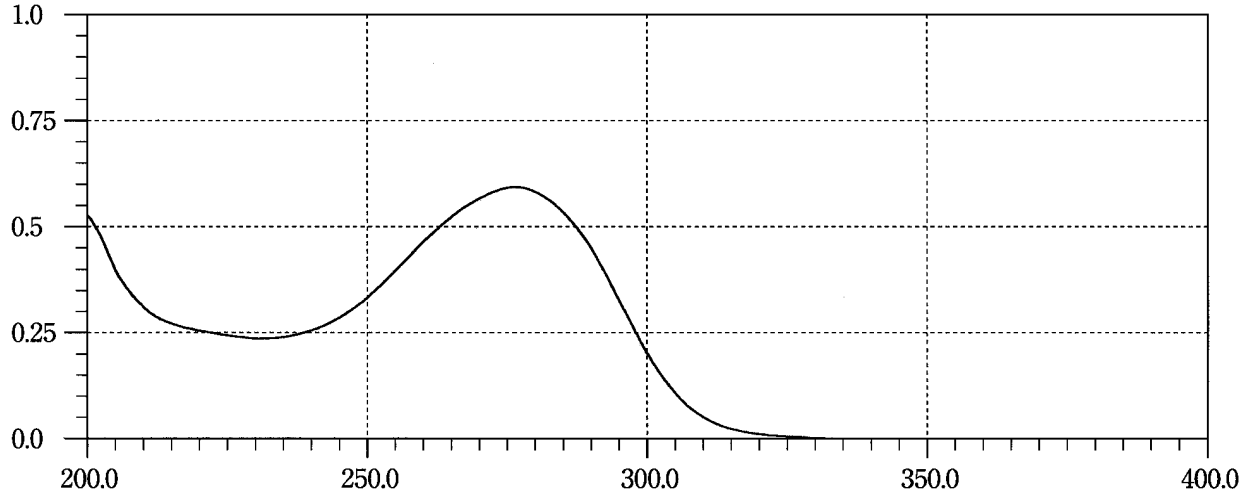
A solution of Cefroxadine in 0.001 mol/L hydrochloric acid TS (1 in 50,000)

Cefteram Pivoxil



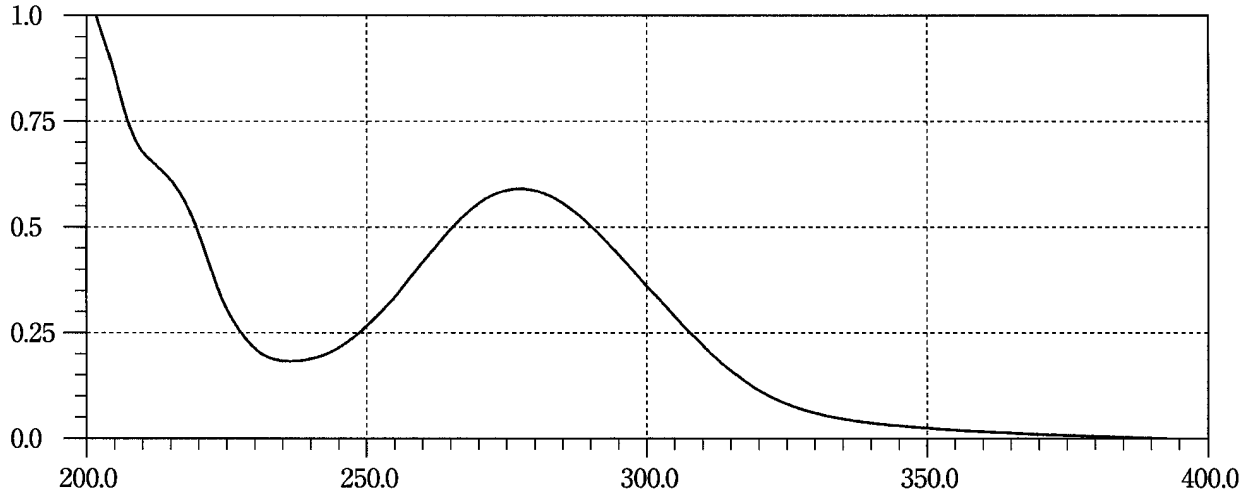
A solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000)

Cefuroxime Axetil



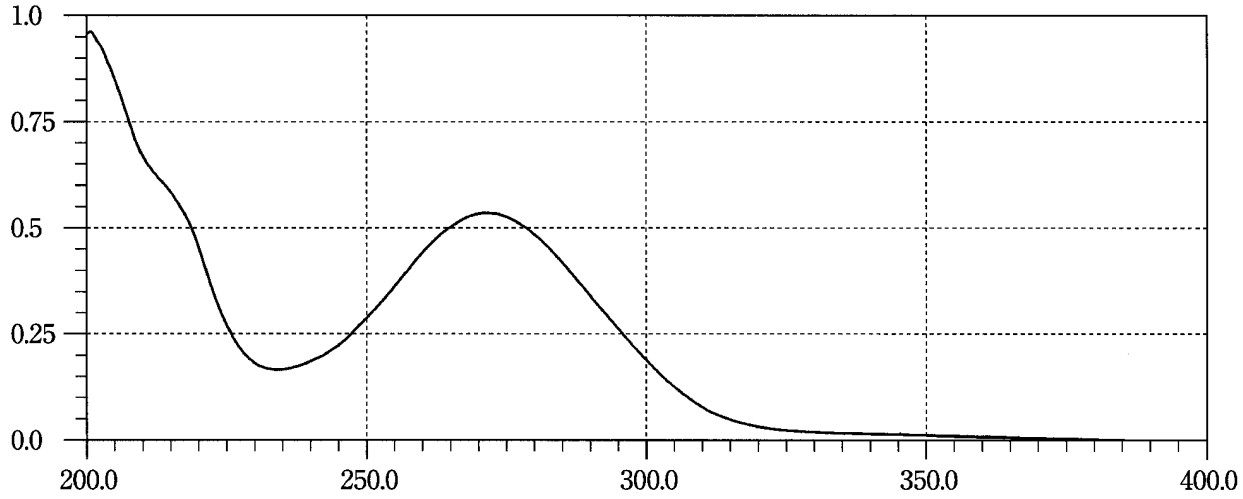
A solution of Cefuroxime Axetil in methanol (3 in 200,000)

Chloramphenicol



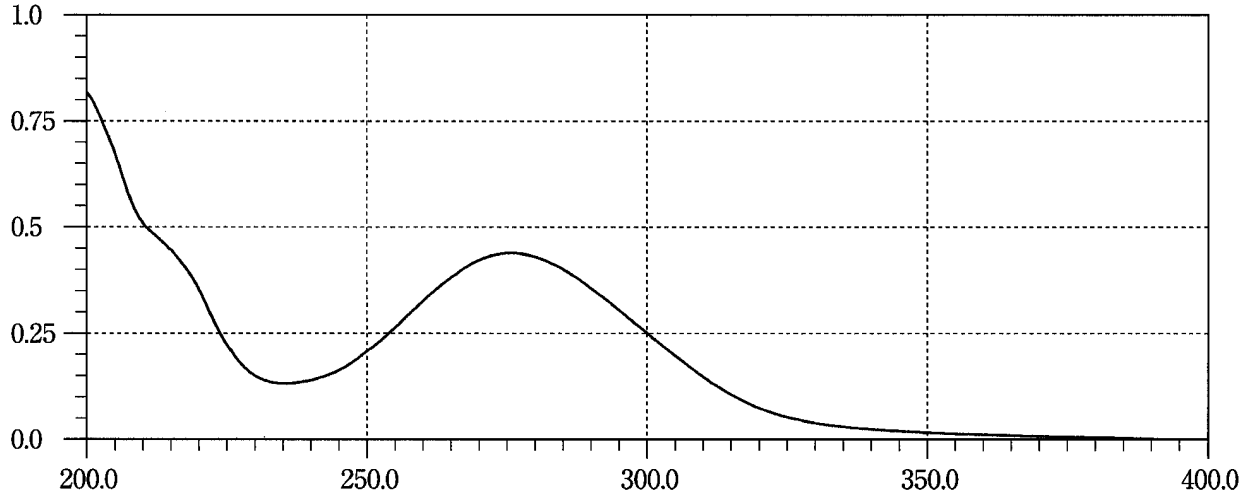
The sample solution obtained in the Assay

Chloramphenicol Palmitate



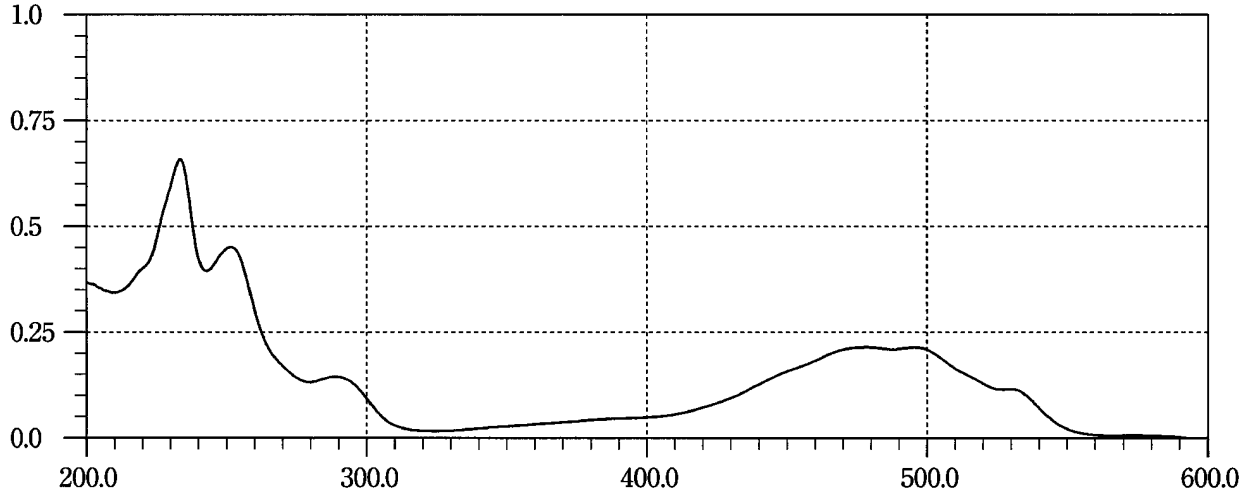
A solution of Chloramphenicol Palmitate in ethanol (99.5) (1 in 33,000)

Chloramphenicol Sodium Succinate



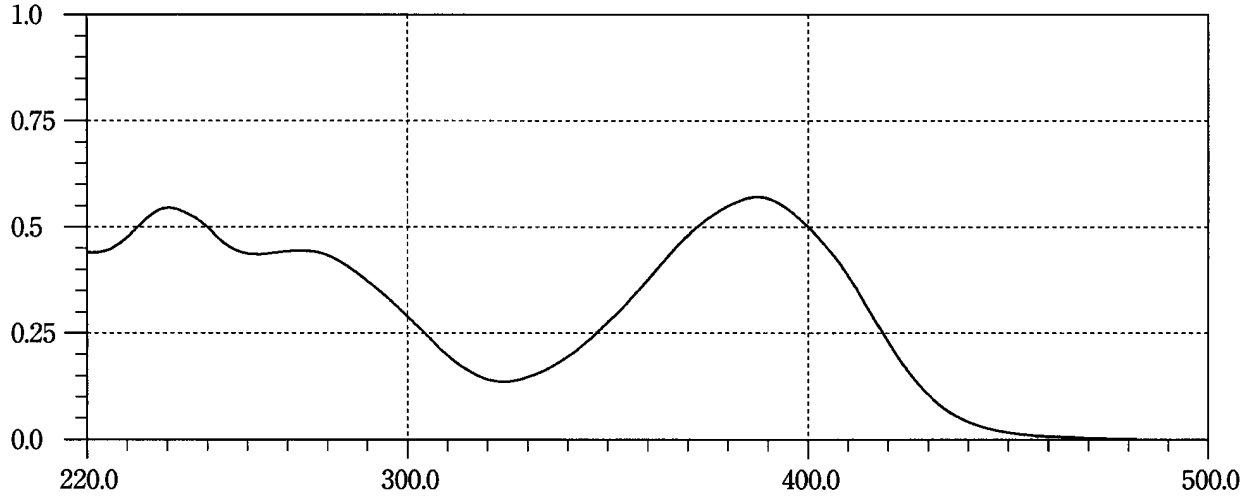
A solution of Chloramphenicol Sodium Succinate (1 in 50,000)

Daunorubicin Hydrochloride



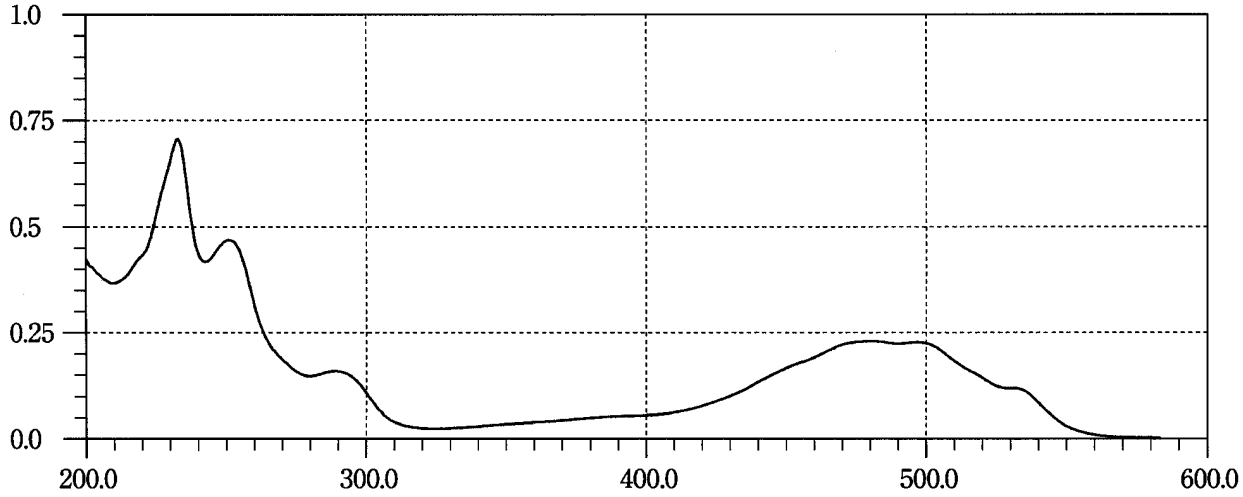
A solution of Daunorubicin Hydrochloride in methanol (1 in 100,000)

Demethylchlortetracycline Hydrochloride



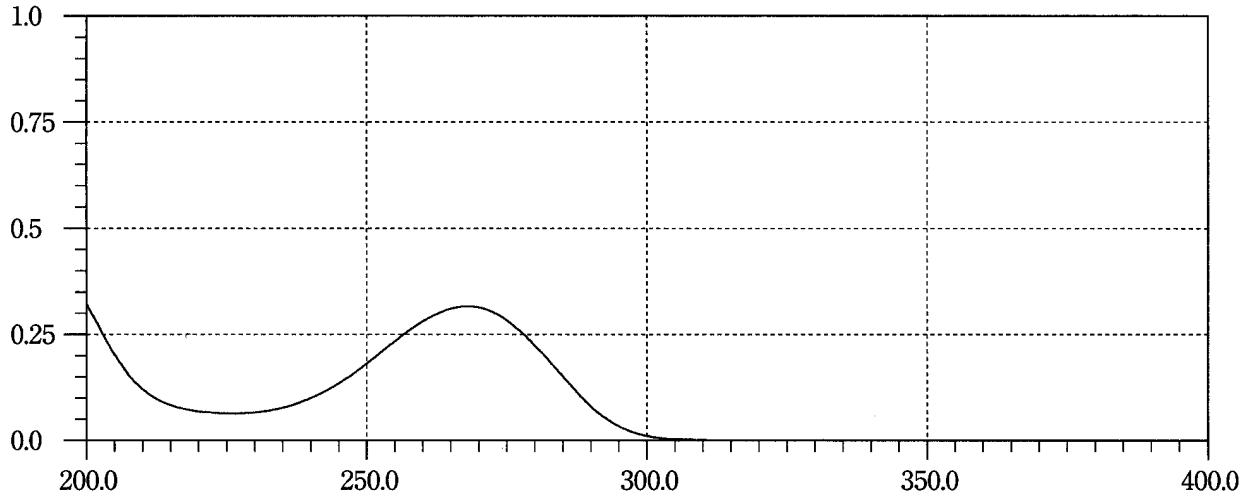
A solution prepared as follows: Dissolve 40 mg of Demethylchlortetracycline Hydrochloride in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5).

Doxorubicin Hydrochloride



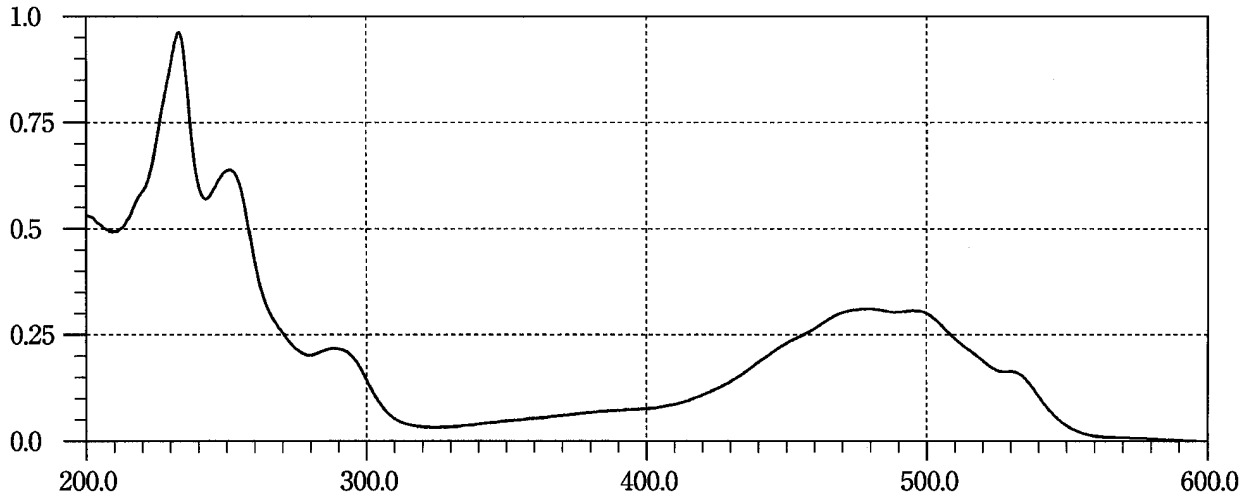
A solution of Doxorubicin Hydrochloride in methanol (1 in 100,000)

Enviomycin Sulfate



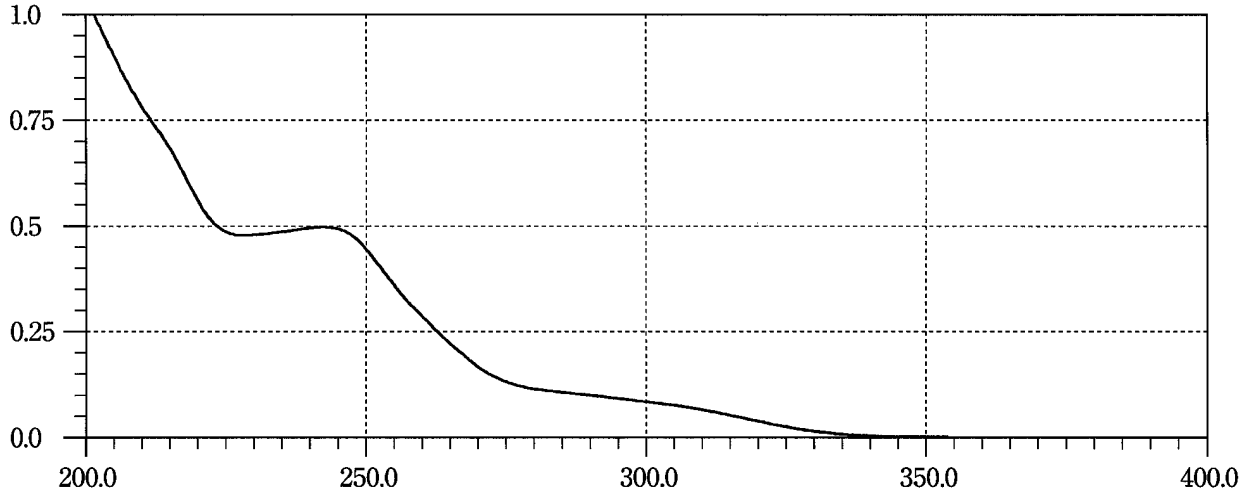
A solution of Enviomycin Sulfate (1 in 100,000)

Epirubicin Hydrochloride



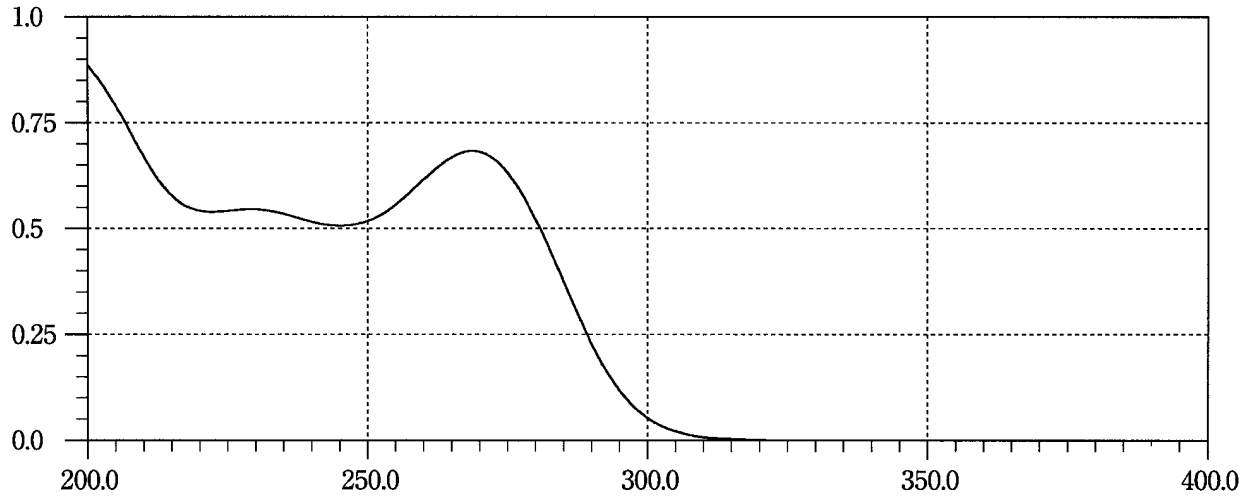
A solution of Epirubicin Hydrochloride in methanol (3 in 200,000)

Etizolam



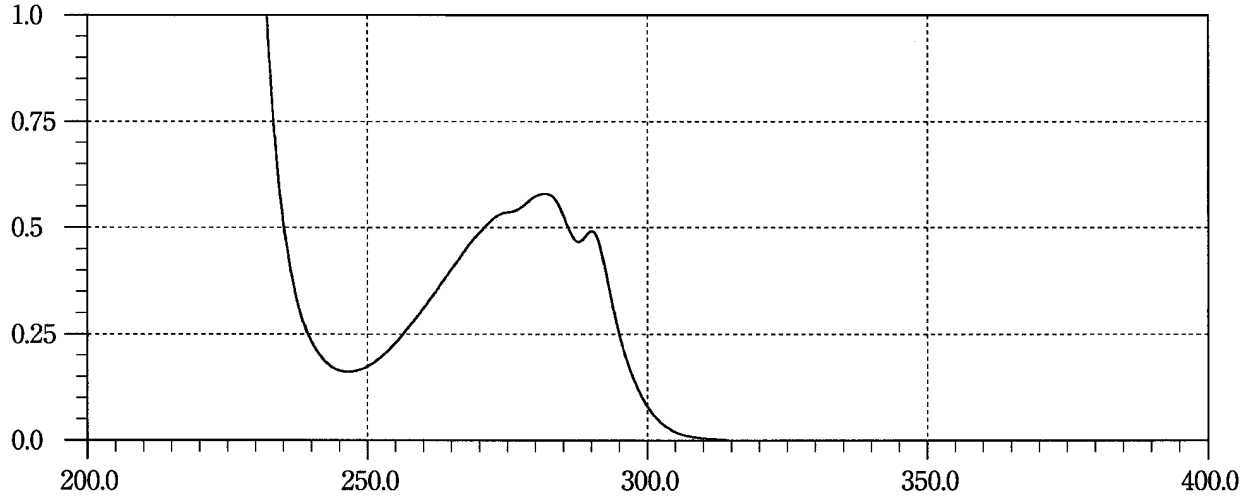
A solution of Etizolam in ethanol (99.5) (1 in 100,000)

Flomoxef Sodium



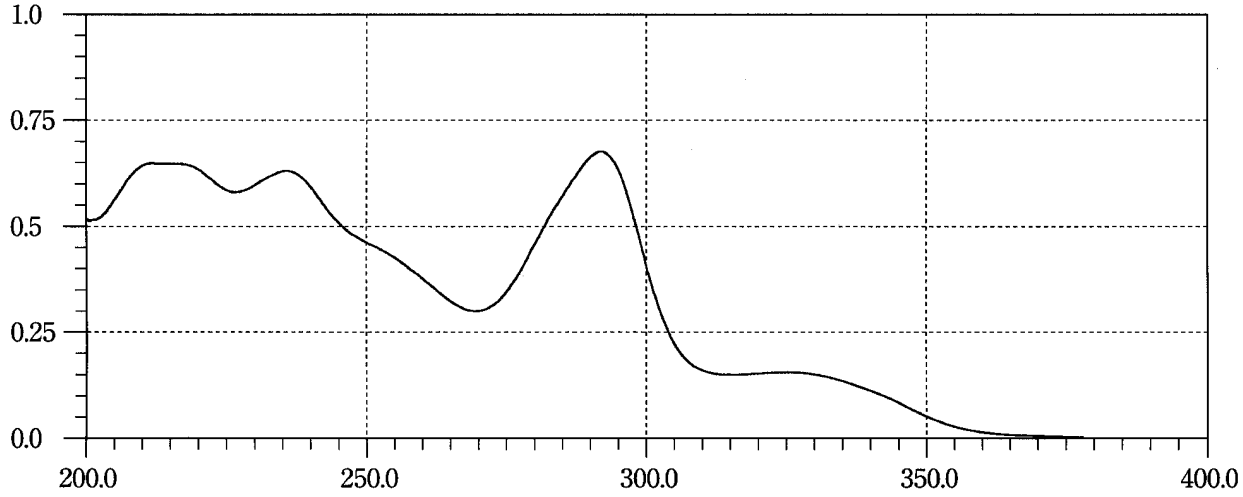
A solution of Flomoxef Sodium (3 in 100,000)

Gramicidin



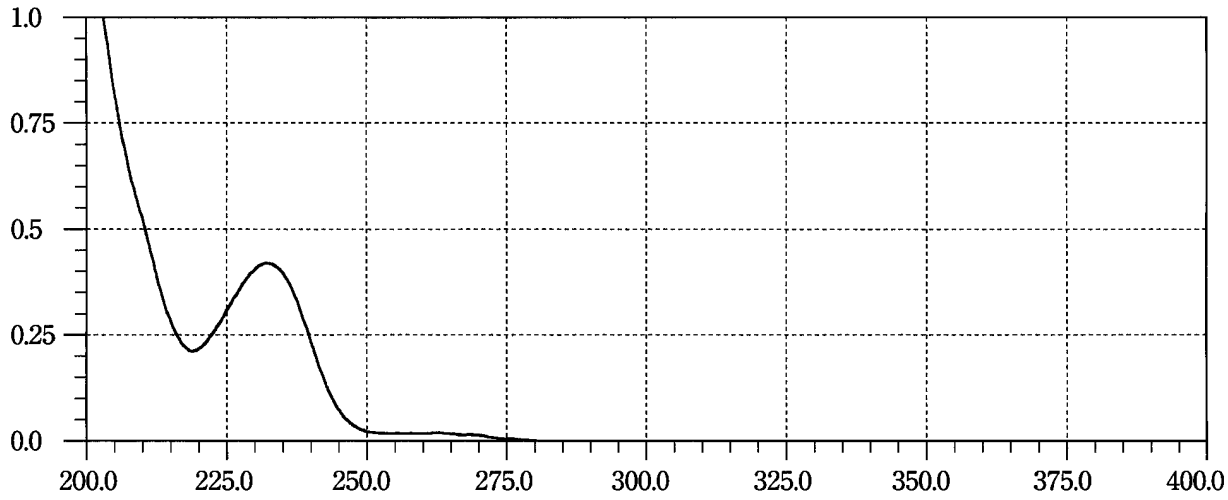
A solution of Gramicidin in ethanol (95) (1 in 20,000)

Griseofulvin



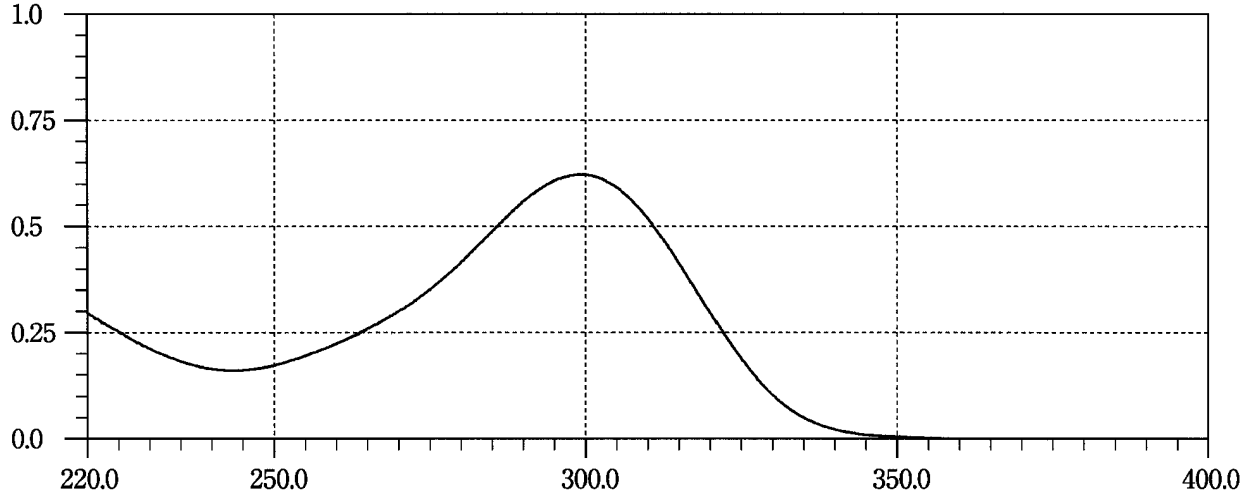
A solution of Griseofulvin in ethanol (95) (1 in 100,000)

Homochlorcyclizine Hydrochloride



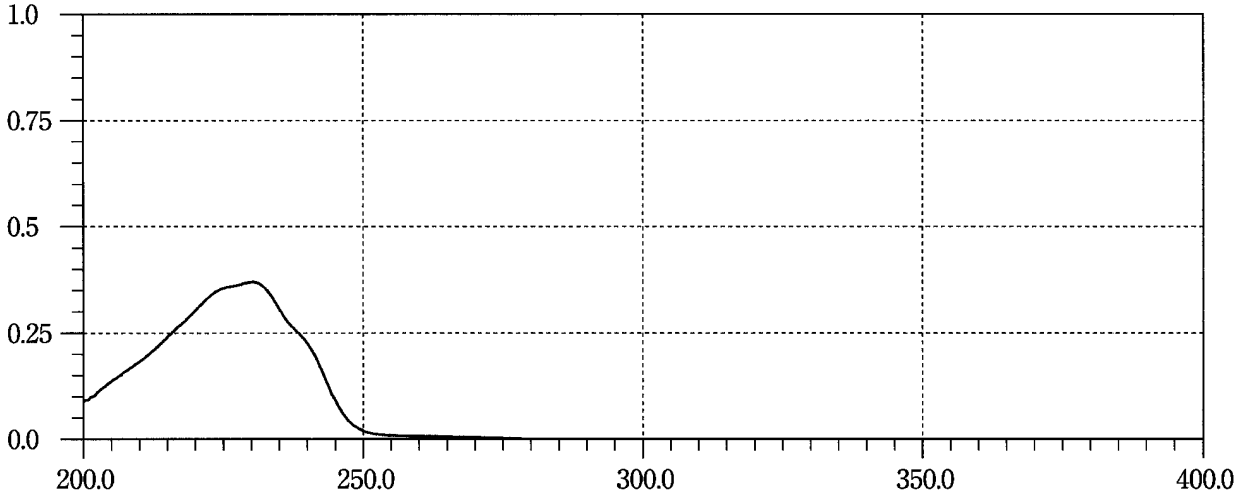
A solution of Homochlorcyclizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000)

Imipenem



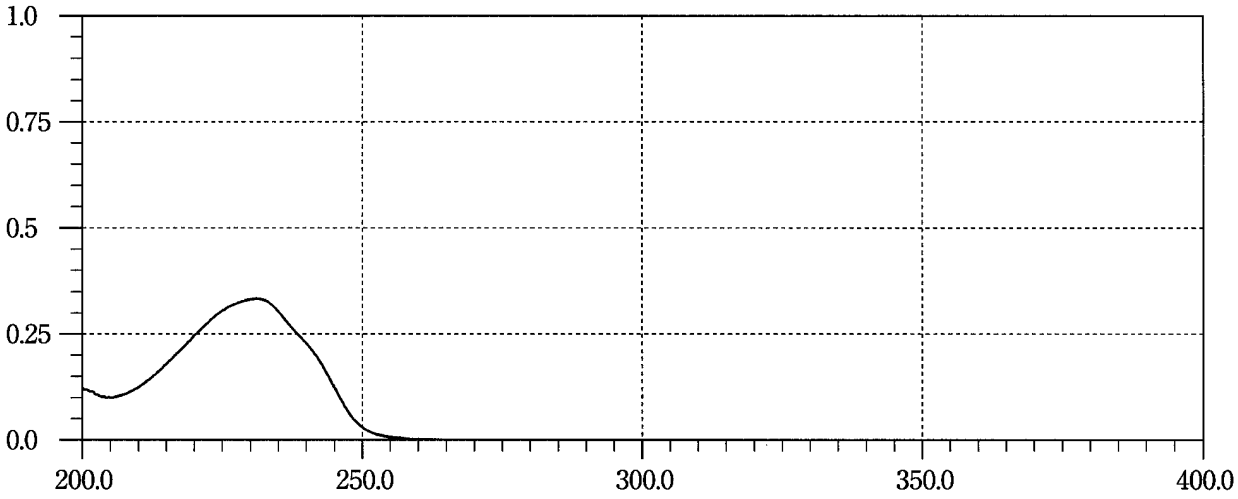
A solution of Imipenem in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000)

Josamycin



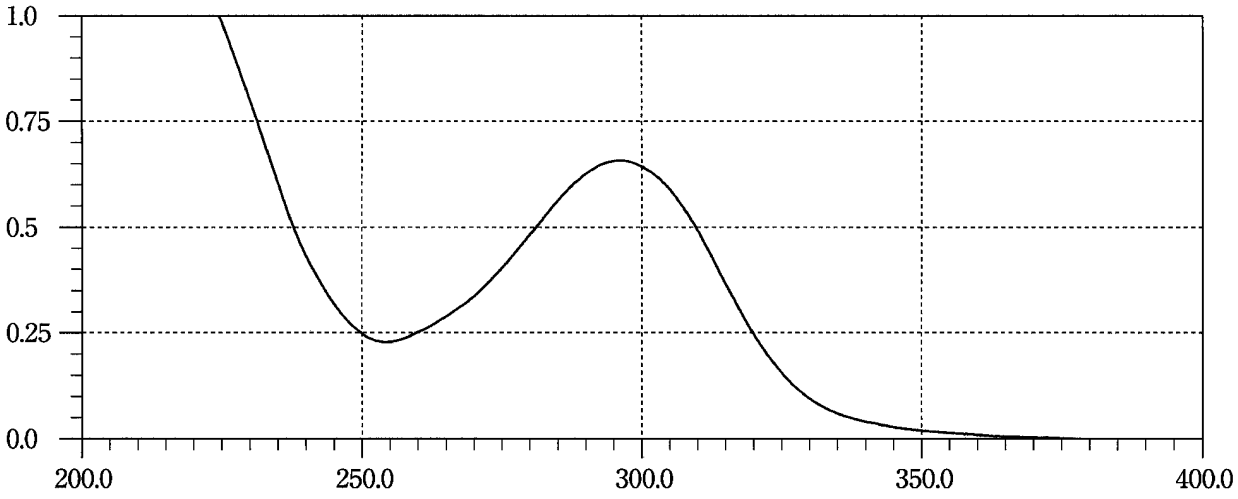
A solution of Josamycin in methanol (1 in 100,000)

Josamycin Propionate



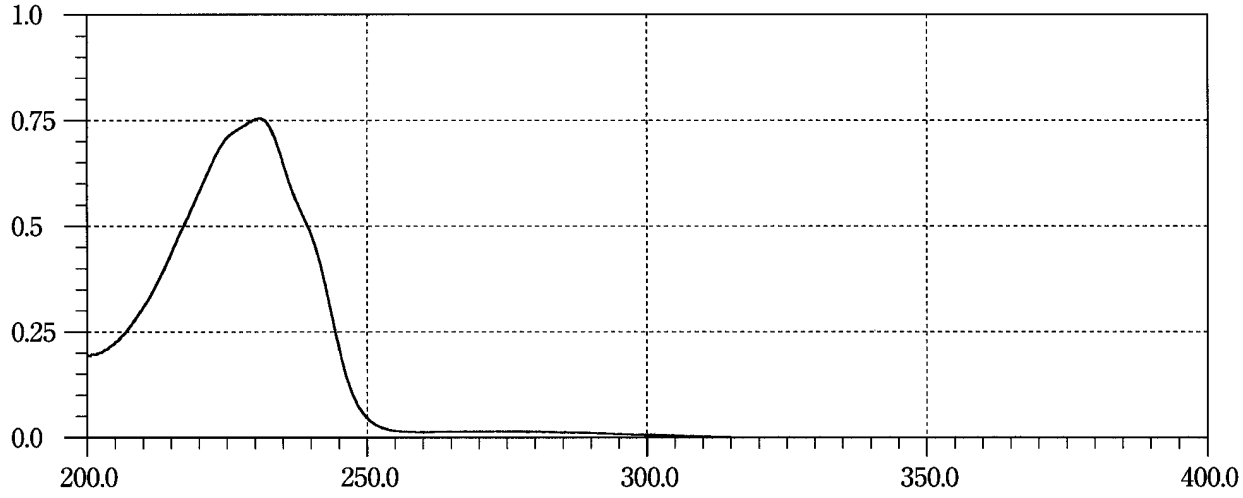
A solution of Josamycin Propionate in methanol (1 in 100,000)

Ketotifen Fumarate



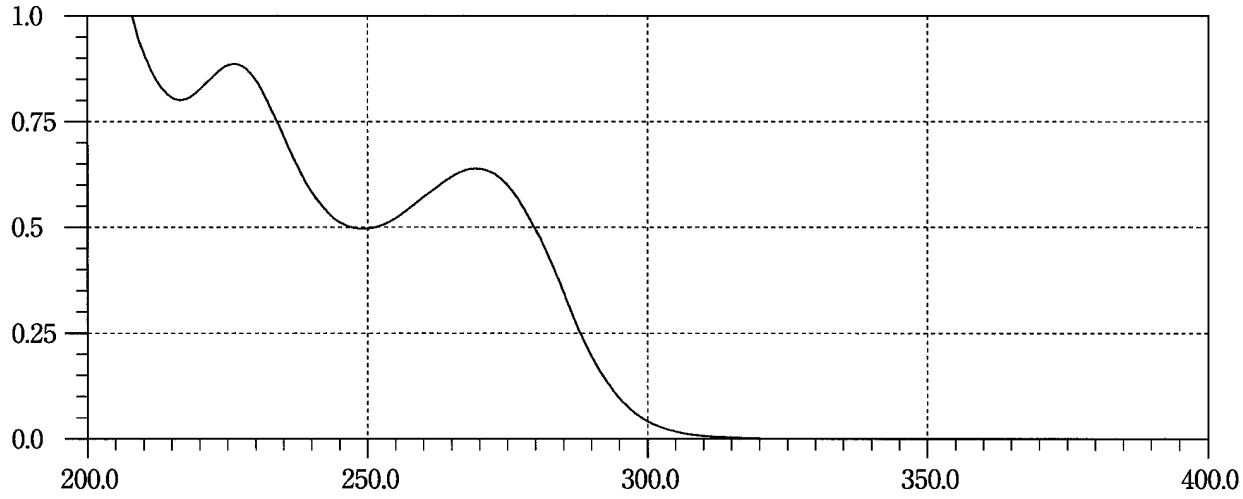
A solution of Ketotifen Fumarate in methanol (1 in 50,000)

Kitasamycin Tartrate



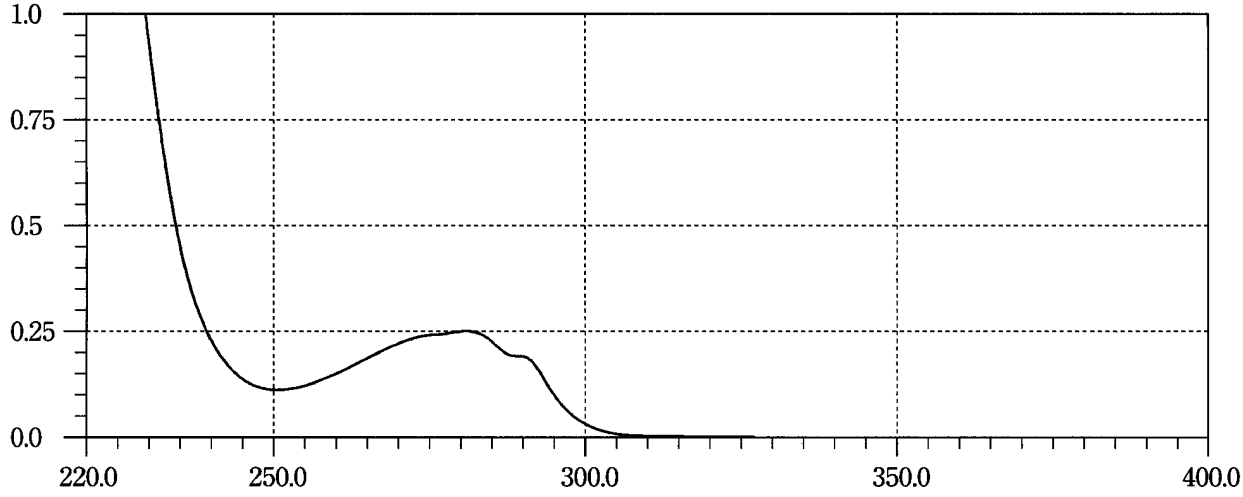
A solution of Kitasamycin Tartrate in methanol (1 in 40,000)

Latamoxef Sodium



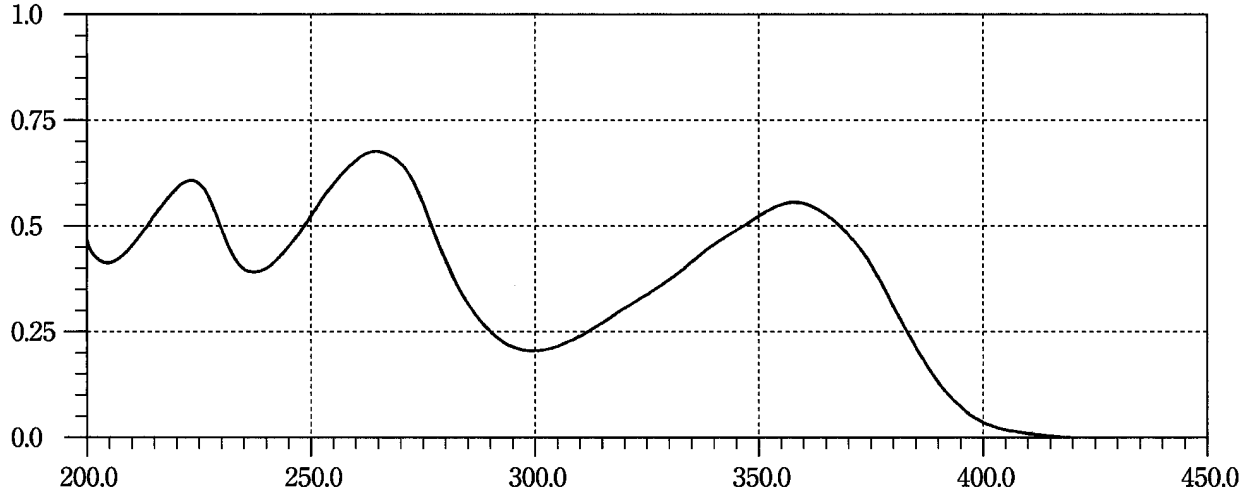
A solution of Latamoxef Sodium (3 in 100,000)

Lysozyme Hydrochloride



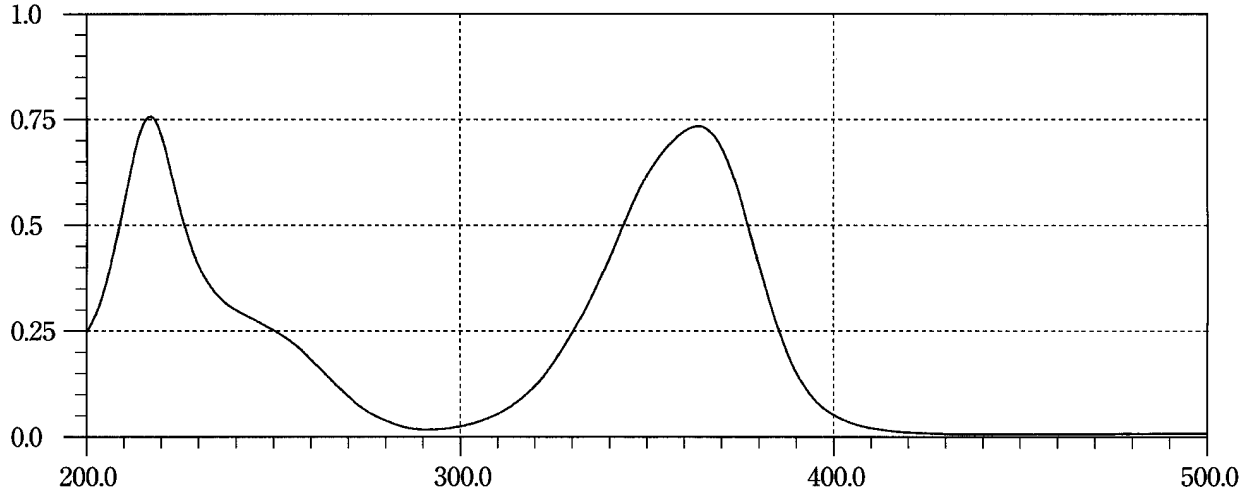
A solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000)

Minocycline Hydrochloride



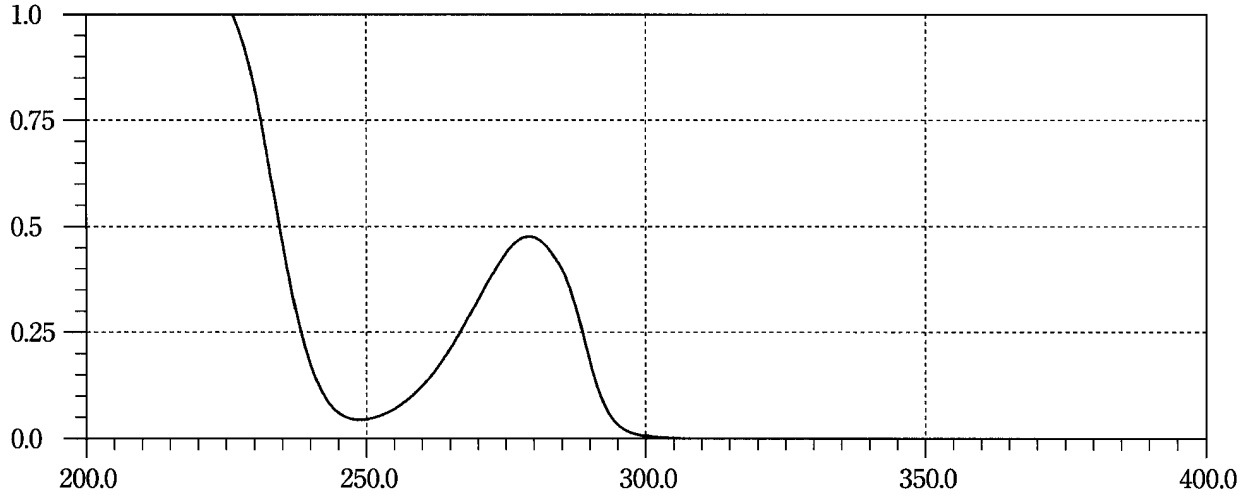
A solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500)

Mitomycin C



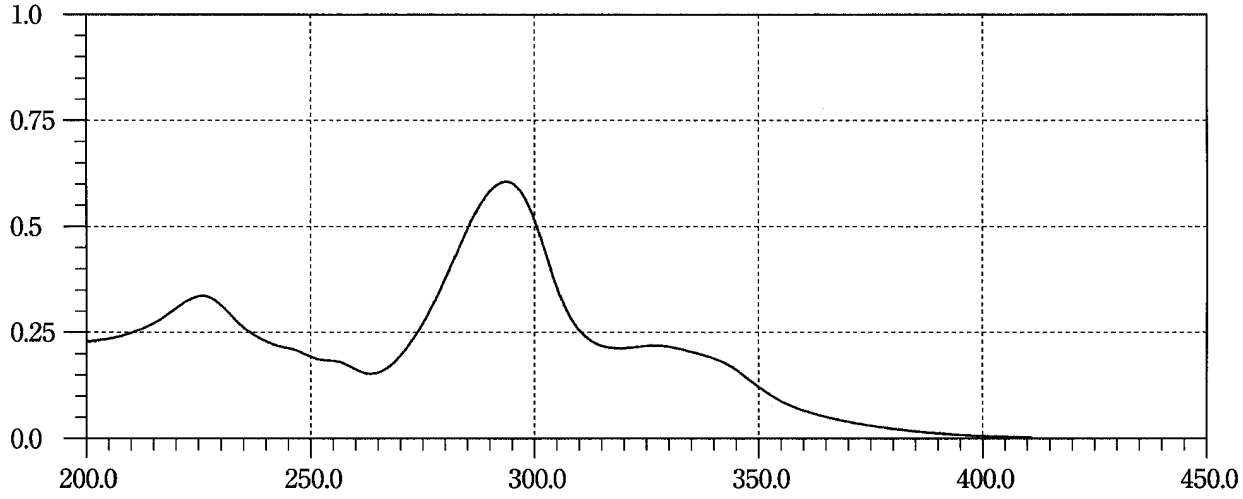
A solution of Mitomycin C (1 in 100,000)

Norepinephrine



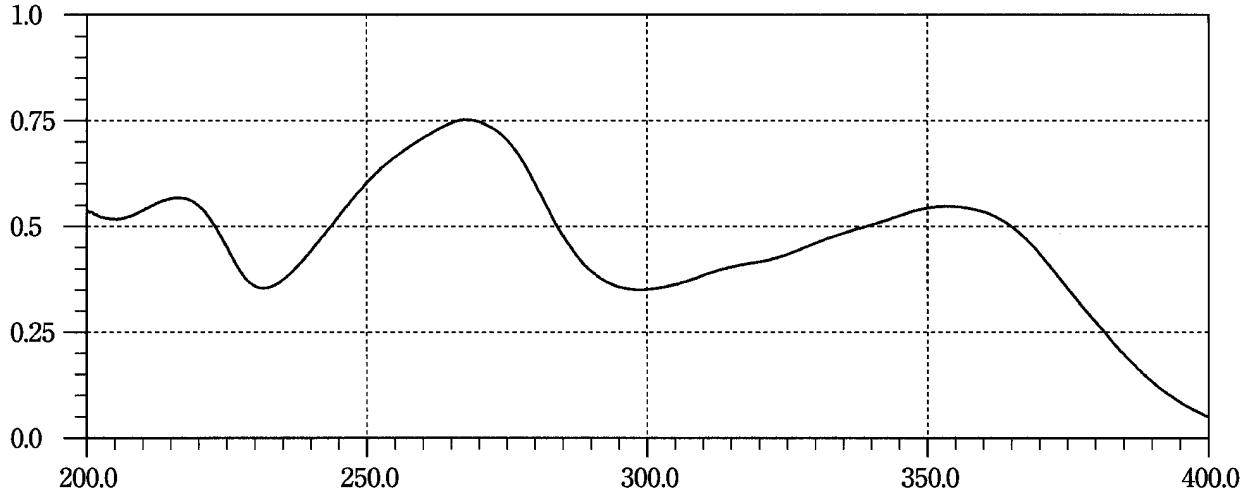
A solution of Norepinephrine in 0.1 mol/L hydrochloric acid TS (3 in 100,000)

Ofloxacin



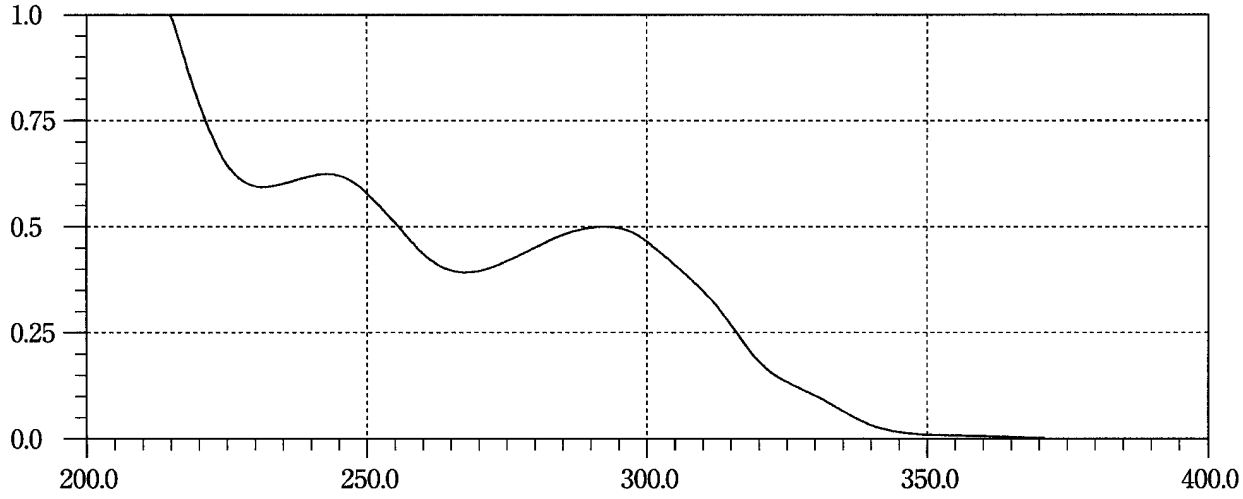
A solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000)

Oxytetracycline Hydrochloride



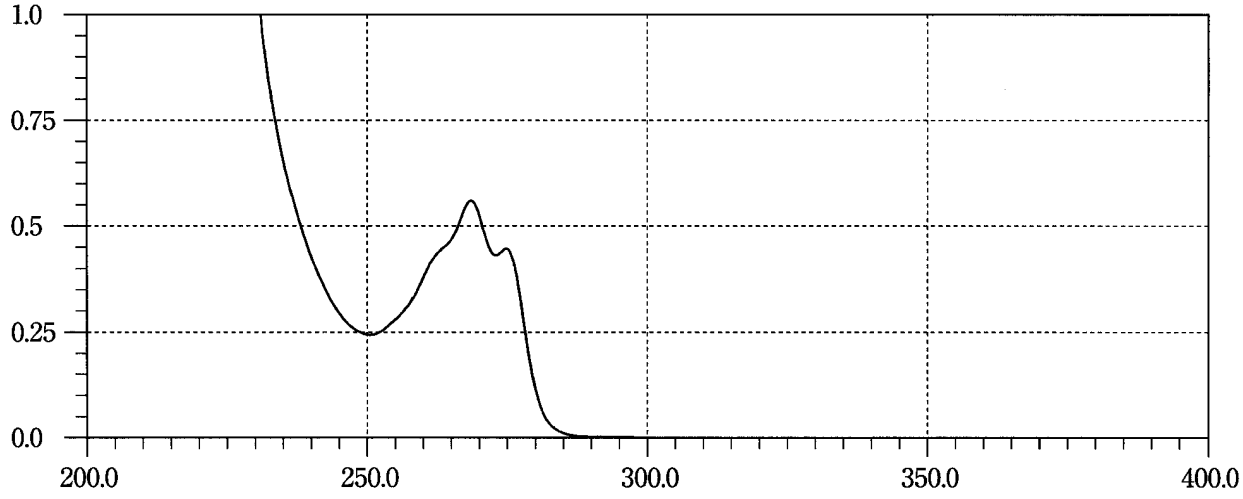
A solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000)

Peplomycin Sulfate



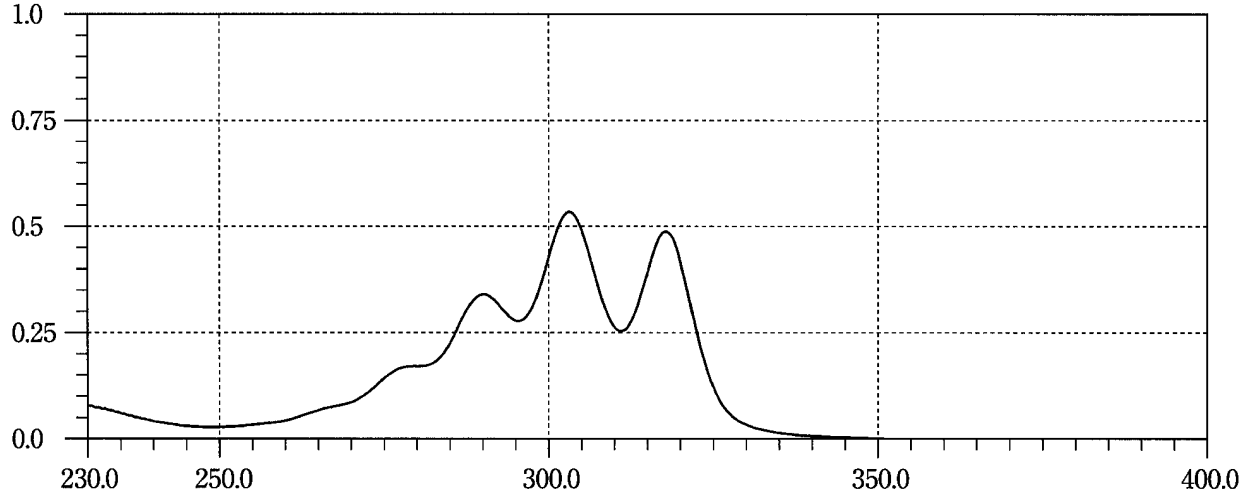
A solution prepared as follows: To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL.

Phenethicillin Potassium



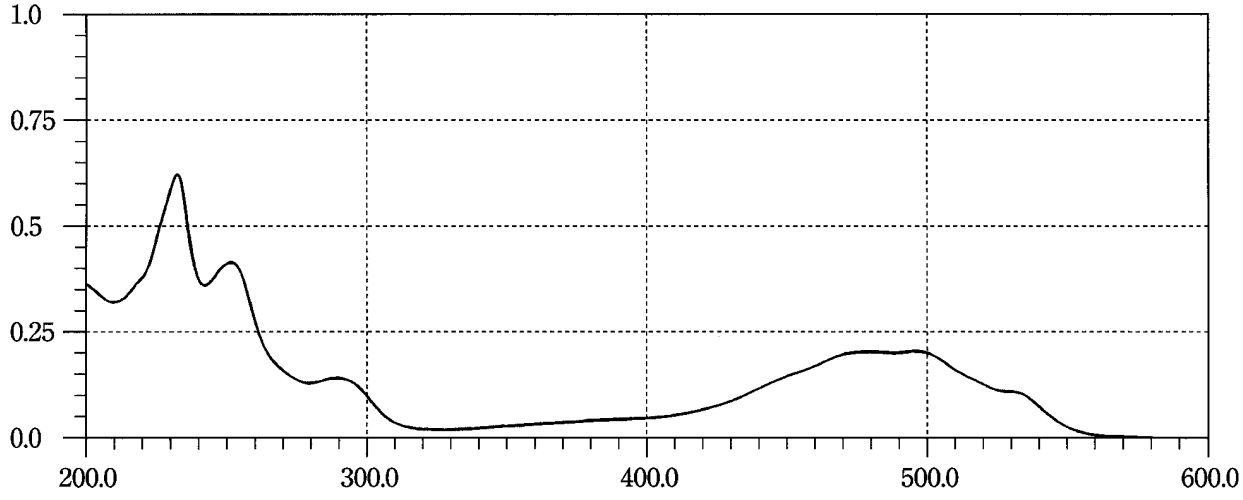
A solution of Phenethicillin Potassium (1 in 5000)

Pimaricin



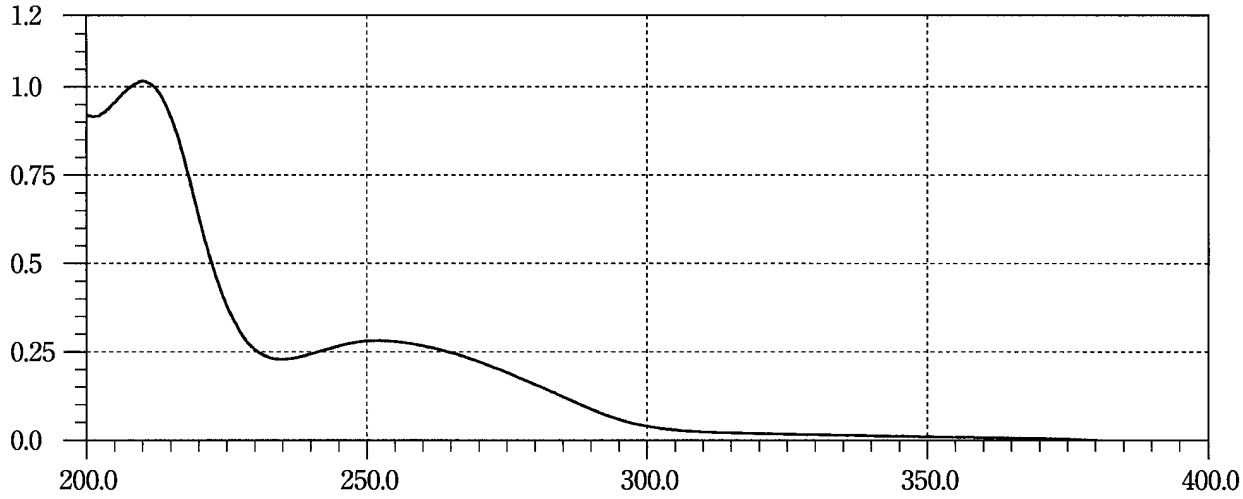
A solution of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) (1 in 200,000)

Pirarubicin



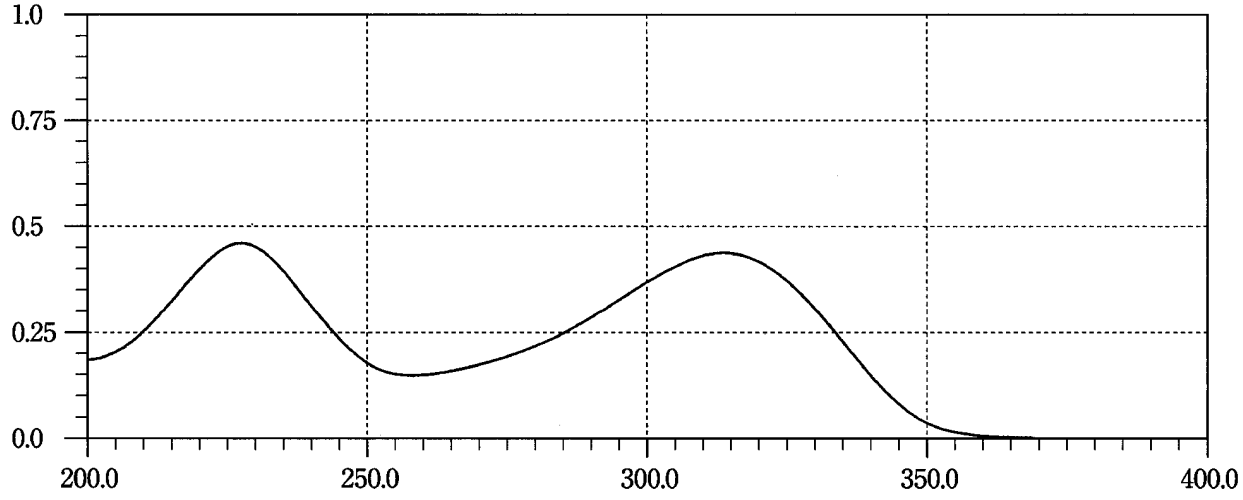
A solution prepared as follows: Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL.

Pyrrolnitrin



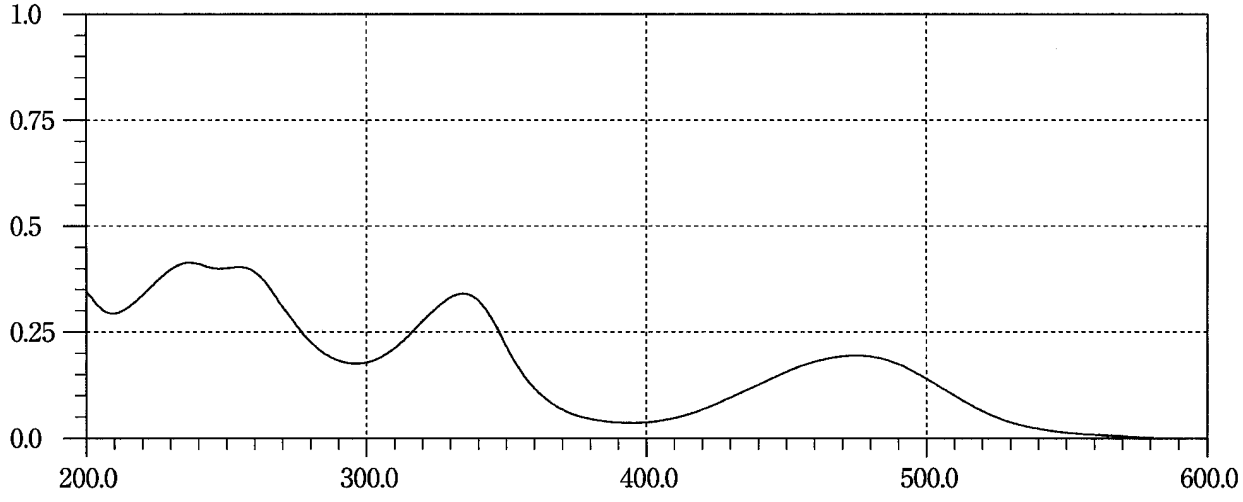
A solution of Pyrrolnitrin in ethanol (95) (1 in 100,000)

Ranitidine Hydrochloride



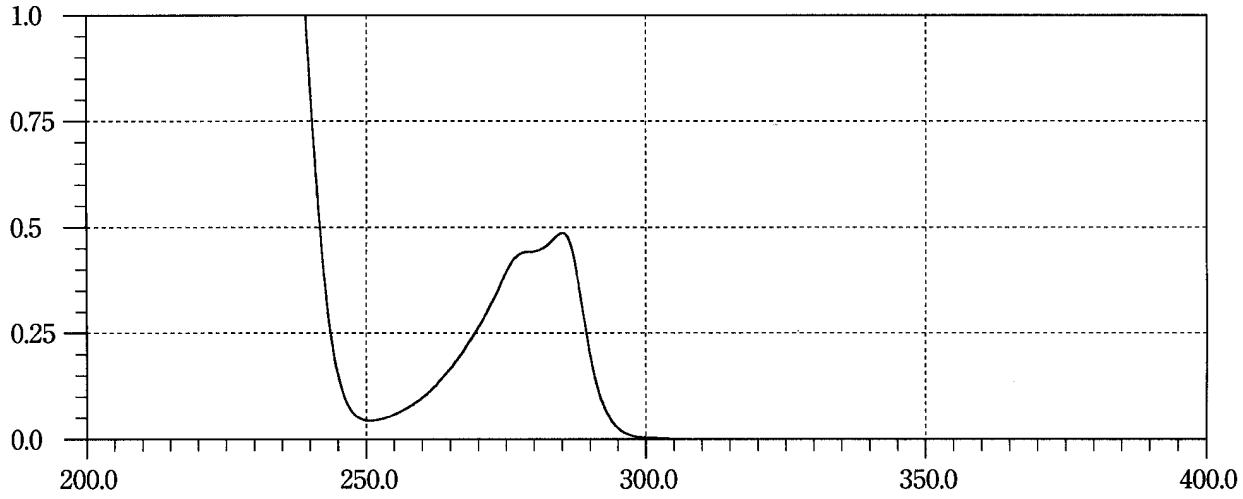
A solution of Ranitidine Hydrochloride (1 in 100,000)

Rifampicin



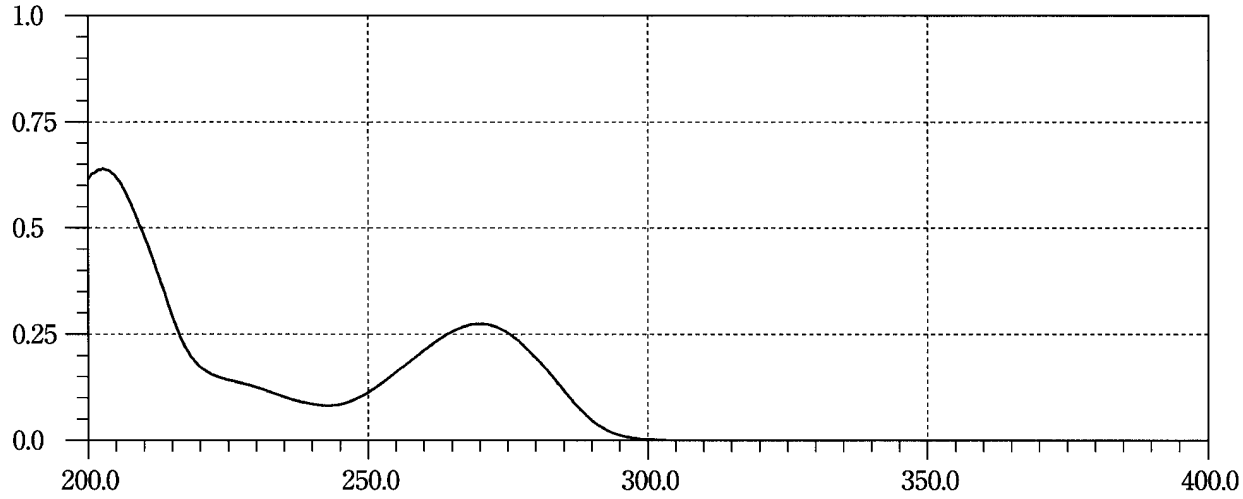
A solution prepared as follows: To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL.

Siccanin



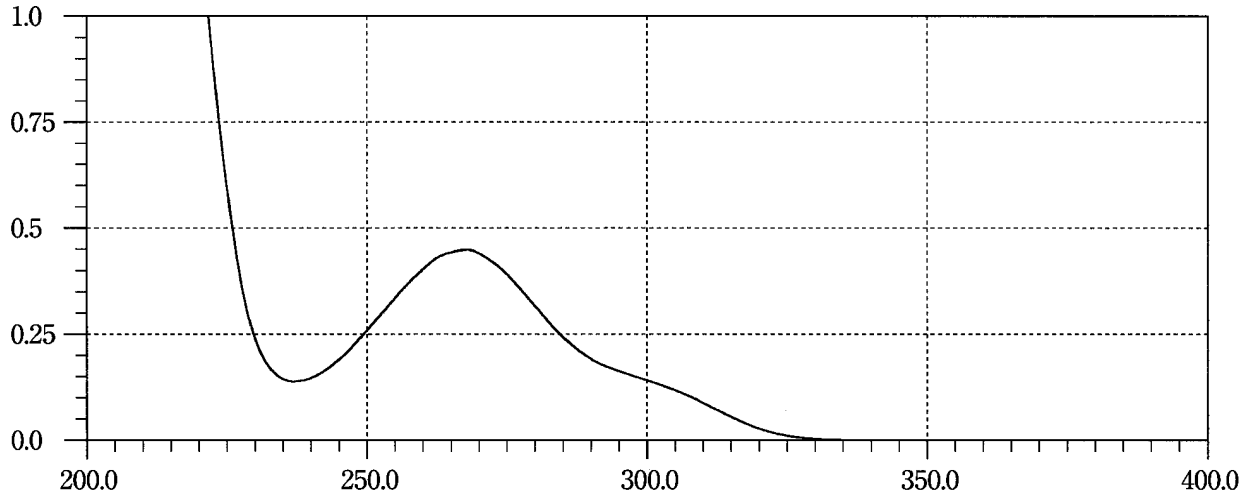
A solution of Siccanin in ethanol (99.5) (1 in 10,000)

Theophylline



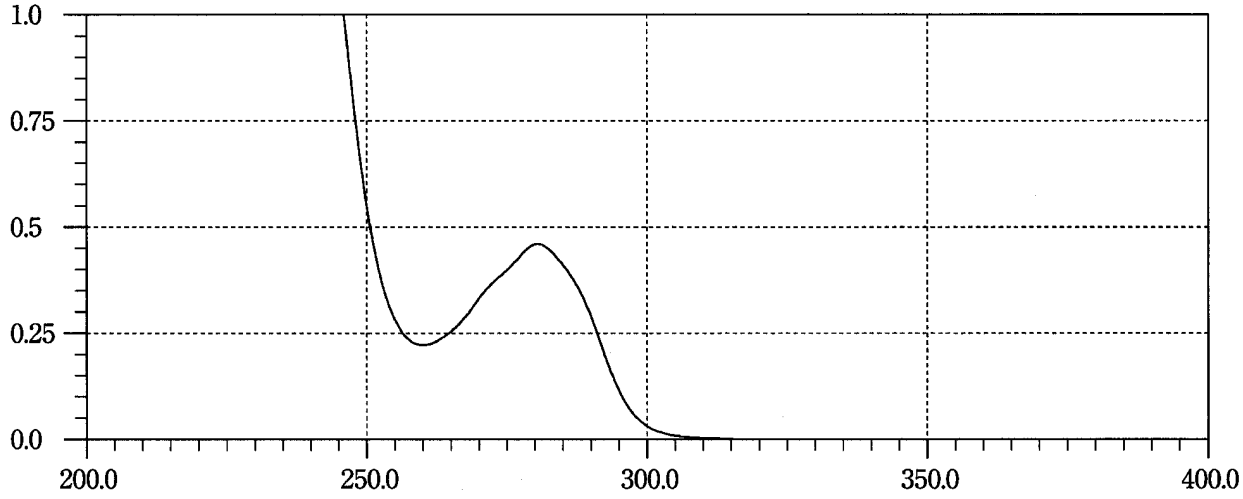
A solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000)

Trimebutine Maleate



A solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000)

Vancomycin Hydrochloride



A solution of Vancomycin Hydrochloride (1 in 10,000)

General Information

Change to read:

1. Aristolochic acid

Aristolochic acid, which occurs in plants of *Aristolochiaceae*, is suspected to cause renal damage. It is also reported to be oncogenic (see References).

Aristolochic acid toxicity will not be a problem if crude drugs of the origin and parts designated in the JP are used, but there may be differences in crude drug nomenclature between different countries, and it is known that crude drug preparations not meeting the specifications of the JP are circulating in some countries. Consequently, when crude drugs or their preparations are used, it is important that the materials should not include any plant containing aristolochic acid.

In the Supplement I to JP14, the test for aristolochic acid I was added to the Purity under Asiasarum Root, which consists of the rhizome and root. Because the aerial part of the plant may contain aristolochic acid and may have been improperly contaminated in Asiasarum Root.

It is considered that Akebia Stem, Sinomenium Stem and Saussurea Root do not contain aristolochic acid, unless plants of origin other than that designated in the JP are used. However, contamination of aristolochic acid might occur, as mentioned above. In this case, the test described in the Purity under Asiasarum Root is useful for checking the presence of aristolochic acid.

References:

Drug & Medical Device Safety Information (No.161) (July, 2000).

New England Journal of Medicine (June 8, 2000).

Mutation Research 515, 63–72 (2002).

Change to read:

5. International Harmonization Implemented in the Japanese Pharmacopoeia Fourteenth Edition

Items for which harmonization has been agreed among the European Pharmacopoeia, the United States Pharmacopeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Fourteenth Edition (JP 14). They are shown in the tables below. The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 14 shows the items as they appear in JP 14. In the Remarks column, notes on any differences between JP 14 and the agreement are shown as occasion demands.

Harmonized items	JP 14	Remarks
Bacterial Endotoxin Test	**Bacterial Endotoxins Test**	
Apparatus	Apparatus	
Preparation of Standard Endotoxin Stock solution	Preparation of Standard Endotoxin Stock Solution	
Preparation of Standard Endotoxin solution	Preparation of Standard Endotoxin Solution	
Preparation of sample solutions	Preparation of sample solutions	
Determination of Maximum Valid Dilution	Determination of Maximum Valid Dilution	
Gel-clot technique (1) Preparatory testing (2) Limit test (3) Assay	Gel-clot techniques (1) Preparatory testing (2) Limit test (3) Assay	
Photometric techniques (1) Turbidimetric technique (2) Chromogenic technique (3) Preparatory testing (4) Assay	Photometric techniques (1) Turbidimetric technique (2) Chromogenic technique (3) Preparatory testing (4) Assay	
Reagents, Test Solutions	**Reagents, Test Solutions**	
Amebocyte lysate	Lysate reagent	
Lysate TS	Lysate TS	
Water for bacterial endotoxins test (BET)	Water for bacterial endotoxins test	

Note: The method for decision of the limit for bacterial endotoxins was agreed between the three pharmacopoeias, but in the Decision of Limit for Bacterial Endotoxins under the General Information in JP 14, the maximum adult dose is calculated based on an average body mass of an adult of 60 kg.

Harmonized items	JP 14	Remarks
Residue on Ignition/Sulphated Ash Test	**Residue on Ignition Test**	
(Introduction)	(Introduction)	Explanation of JP's particular expressions in the introduction: The description, for example, ''not more than 0.10 % (1 g),'' in a monograph, indicates that the mass of the residue is not more than 1.0 mg per g of the substance to be tested in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and ''after drying'' indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying.
Procedure	Procedure	Explanation of JP's particular expressions for taking the sample: When the quantity of the sample to be taken is indicated in a volume, pipet exactly the amount directed in the monograph and transfer to the above crucible. When directed as ''after evaporating,'' heat properly to evaporate the solution.

Harmonized items	JP 14	Remarks
Sodium Chloride	**Sodium Chloride**	
Definition	limits of the content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Bromides	Purity (5) Bromides	
Ferrocyanides	Purity (7) Ferrocyanides	
Iodides	Purity (6) Iodides	
Nitrites	not specified	
Phosphates	Purity (4) Phosphates	
Sulphates	Purity (3) Sulfates	
Aluminium	not specified	
Barium	Purity (10) Barium	
Iron	Purity (9) Iron	
Magnesium and alkaline-earth metals	Purity (11) Magnesium and alkaline-earth materials	
Potassium	not specified	
Loss on drying	Loss on drying	
Bacterial endotoxins	not specified	
Assay	Assay	

Harmonized items	JP 14	Remarks
Citric Acid Monohydrate	**Citric Acid**	
Definition	limits of the content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalate	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Bacterial endotoxins	not specified	
Assay	Assay	
Storage	Containers and storage	

Harmonized items	JP 14	Remarks
Citric Acid, Anhydrous	**Anhydrous Citric Acid**	
Definition	limits of the content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalate	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Bacterial endotoxins	not specified	
Assay	Assay	
Storage	Containers and storage	

Harmonized items	JP 14	Remarks
General Information	**General Information**	
Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	**SDS-Polyacrylamide Gel Electrophoresis**	
Characteristics of Polyacrylamide Gels	1. Characteristics of Polyacrylamide Gels	
Denaturing Polyacrylamide Gel Electrophoresis	2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions	
Reducing conditions	1) Reducing conditions	
Non-reducing conditions	2) Non-reducing conditions	
Characteristics of Discontinuous Buffer System Gel Electrophoresis	3. Characteristics of Discontinuous Buffer System Gel Electrophoresis	
Preparing Vertical Discontinuous Buffer SDS Polyacrylamide Gels	4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels	
Assembling of the gel moulding cassette	1) Assembling of the gel moulding cassette	
Preparation of the gel	2) Preparation of the gel	
Mounting the gel in the electrophoresis apparatus and electrophoretic separation	3) Mounting the gel in the electrophoresis apparatus and electrophoretic separation	
Detection of Protein in Gels	5. Detection of Proteins in Gels	
Coomassie staining	1) Coomassie staining	
Silver staining	2) Silver staining	
Drying of Stained SDS Polyacrylamide Gels	6. Drying of Stained SDS-Polyacrylamide Gels	
Molecular-Mass Determination	7. Molecular-Mass Determination	
Validation of the Test	8. Suitability of the Test	
Quantification of Impurities	9. Quantification of Impurities	
Reagents, Test Solutions	Test Solutions	
Blocking solution	Blocking TS	
Coomassie staining solution	Coomassie staining TS	
Destaining solution	Destaining TS	
Developer solution	Developer TS	
Fixing solution	Fixing TS	
Silver nitrate reagent	Silver nitrate TS for silver staining	
Trichloroacetic acid reagent	Trichloroacetic acid TS for fixing	
Table 1 − Preparation of resolving gel	Table 1. Preparation of resolving gel	
Table 2 − Preparation of stacking gel	Table 2. Preparation of stacking gel	

Change to read:

12. Preservatives-Effectiveness Tests

The purpose of the Preservatives-Effectiveness Tests is to assess microbiologically the preservative efficacy, either due to the action of product components themselves or any added preservative(s), for multi-dose containers. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and titration of survival of the test strains with time.

Preservatives must not be used solely to comply with GMP for drugs or to reduce viable aerobic counts. In addition, preservatives themselves are toxic substances. Therefore, preservatives must not be added to products in amounts which might jeopardize the safety of human beings, and consideration must be given to minimizing the amounts of preservative used. These tests are commonly used to verify that products maintain their preservative effectiveness at the design phase of formulation or in the case of periodic monitoring. Although these tests are not performed for lot release testing, the efficacy of the preservative present in the product packaged in the final containers should be verified throughout the entire dating period.

1. Products and their Categories

The products have been divided into two categories for these tests. Category I products are those made with aqueous bases or vehicles, and Category II products are those made with nonaqueous bases or vehicles. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II products. Category I is further divided into three subtypes depending on the dosage forms.

Category IA: Injections and other sterile parenterals

Category IB: Nonsterile parenterals

Category IC: Oral products made with aqueous bases (including syrup products to be dissolved or suspended before use)

Category II: All the dosage forms listed under Category I made with nonaqueous bases or vehicles.

2. Test Microorganisms and Culture Media

The following strains or those considered to be equivalent are used as the test microorganisms.

Escherichia coli ATCC 8739, NBRC 3972

Pseudomonas aeruginosa ATCC 9027, NBRC 13275

Staphylococcus aureus ATCC 6538, NBRC 13276

Candida albicans ATCC 10231, NBRC 1594, JCM 2085

Aspergillus niger ATCC 16404, NBRC 9455

These test microorganisms are representative of those that might be found in the environment in which the product is manufactured, used or stored, and they are also recognized as opportunistic pathogens. In addition to these strains designated as test microorganisms, it is further recommended to use strains that might contaminate the product and grow on or in it, depending on its characteristics. For the test microorganisms received from coordinated collections of microorganisms, one passage is defined as the transfer of microorganisms from an established culture to fresh medium, and microorganisms subjected to not more than five passages should be used for the tests. Single-strain challenges rather than mixed cultures should be used. The test strains can be harvested by growth on solid agar or liquid media.

Cultures on agar plate media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Agar, Glucose-Peptone Agar or Potato Dextrose Agar Medium. Incubate bacterial cultures at 30°C to 35°C for 18 to 24 hours, the culture of *C. albicans* at 20°C to 25°C for 40 to 48 hours and the culture of *A. niger* at 20°C to 25°C for one week or until good sporulation is obtained. Harvest these cultured cells aseptically using a platinum loop, etc. Suspend the collected cells in sterile physiological saline or in 0.1% peptone water and adjust the viable cell count to about 10^8 microorganisms per mL. In the case of *A. niger*, suspend the cultured cells in sterile physiological saline or 0.1% peptone water containing 0.05 w/v% of polysorbate 80 and adjust the spore count to about 10^8 per mL. Use these suspensions as the inocula.

Liquid cultures: After culturing each of the four strains except for *A. niger* in a suitable medium, remove the medium by centrifugation. Wash the cells in sterile physiological saline or 0.1% peptone water and resuspend them in the same solution with the viable cell or spore count of the inoculum adjusted to about 10^8 per mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. Use the inoculum suspensions within 24 hours after they have been prepared from the cultivations on agar plate media or in liquid media. Store the inoculum suspensions in a refrigerator if it is not possible to inoculate them into the test specimens within 2 hours. Titrate the viable cell count of the inocula immediately before use, and then calculate the theoretical viable cell count per mL (g) of the product present just after inoculation.

3. Test Procedure

3.1 Category I products

Inject each of the cell suspensions aseptically into five containers containing the product and mix uniformly. When it is difficult to inject the cell suspension into the container aseptically or the volume of the product in each container is too small to be tested, transfer aseptically a sufficient volume of the product into each of alternative sterile containers, and mix the inoculum. When the product is not sterile, incubate additional containers containing the uninoculated product as controls and calculate their viable cell counts (the viable counts of bacteria and those of yeasts and moulds). A sterile syringe, spatula or glass rod may be used to mix the cell suspension uniformly in the product. The volume of the suspension mixed in the product must not exceed 1/100 of the volume of the product. Generally, the cell suspension is in-

oculated and mixed so that the concentration of viable cells is 10^5 to 10^6 cells per mL or per gram of the product. Incubate these inoculated containers at 20°C to 25°C with protection from light, and calculate the viable cell count of 1 mL or 1 g of the product taken at 0, 14 and 28 days subsequent to inoculation. Record any marked changes (e.g., changes in color or the development of a bad odor) when observed in the mixed samples during this time. Such changes should be considered when assessing the preservative efficacy of the product concerned. Express sequential changes in the viable counts as percentages, with the count at the start of the test taken as 100. Titration of the viable cell counts is based, in principle, on the Pour Plate Methods in ''Microbial Limit Tests''. In this case, confirm whether any antimicrobial substance is present in the test specimen. If a confirmed antimicrobial substance needs to be eliminated, incorporate an effective inactivator of the substance in the buffer solution or liquid medium to be used for dilution of the test specimen, as well as in the agar plate count medium. However, it is necessary to confirm that the inactivator has no effect on the growth of the microorganisms. When the occurrence of the preservative or the product itself affects titration of the viable cell count and there is no suitable inactivator available, calculate the viable cell counts by the Membrane Filtration Method in ''Microbial Limit Tests''.

3.2 Category II products

The procedures are the same as those described for Category I products, but special procedures and considerations are required for both uniform dispersion of the test microorganism in the product and titration of viable cell counts in the samples.

For semisolid ointment bases, heat the sample to 45°C to 50°C until it becomes oily, add the cell suspension and disperse the inoculum uniformly with a sterile glass rod or spatula. Surfactants may also be added to achieve uniform dispersion, but it is necessary to confirm that the surfactant added has no effect on survival or growth of the test microorganisms and that it does not potentiate the preservative efficacy of the product. For titration of the viable cell count, a surfactant or emulsifier may be added to disperse the product uniformly in the buffer solution or liquid medium. Sorbitan monooleate, polysorbate 80 or lecithin may be added to improve miscibility between the liquid medium and semisolid ointments or oils in which test microorganisms were inoculated. These agents serve to inactivate or neutralize many of the most commonly used preservatives.

4. Interpretation

Interpret the preservative efficacy of the product according to Table 1. When the results described in Table 1 are obtained, the product examined is considered to be effectively preserved. There is a strong possibility of massive microbial contamination having occurred when microorganisms other than the inoculated ones are found in the sterile product to be examined, and caution is required in the test procedures and/or the control of the manufacturing process of the product. When the contamination level in a nonsterile product to be examined exceeds the microbial enumeration limit specified in ''Microbial Attributes of Nonsterile Pharmaceutical Products'' in General Information, caution is also required in the test procedures and/or the control of the manufacturing process of the product.

Table 1. Interpretation criteria by product category

Product category	Microorganisms	Interpretation criteria	
		After 14 days	After 28 days
Category IA	Bacteria	0.1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category IB	Bacteria	1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category IC	Bacteria	10% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category II	Bacteria	Same or less than inoculum count	Same or less than inoculum count
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count

5. Culture Media

Culture media and buffer solution used for Preservatives-Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

Soybean Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 7.1 – 7.3.

Sabouraud Glucose Agar Medium

Peptone (animal tissue and casein)	10.0 g
Glucose	40.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 –

20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

Glucose Peptone (GP) Agar Medium

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Peptone	5.0 g
Monobasic potassium phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 5.6 – 5.8.

Potato Dextrose Agar Medium

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

0.1% Peptone Water

Peptone	1.0 g
Sodium chloride	8.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 7.2 – 7.4.

Add the following:

17. Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia

Introduction

The primary role of specification of biotechnological/biological products listed in Japanese Pharmacopoeia (JP) is not only for securing quality control or consistency of the quality but also for assuring their efficacy and safety. In the meantime, the requirements to assure quality and safety of drugs have come to be quite strict recently, and a rigid attitude addressing safety assurance is expected for biotechnological/biological products. The key points for quality and safety assurance of biotechnological/biological products are selection and appropriate evaluation of source material, appropriate evaluation of manufacturing process and maintenance of manufacturing consistency, and control of specific physical properties of the products. Now, how to assure quality and safety of such drugs within a scope of JP has come to be questioned. This General Information describes what sorts of approaches are available to overcome these issues.

It is desired that quality and safety assurance of JP listed products are achieved by state-of-the-art methods and concepts which reflect progress of science and accumulation of experiences. This General Information challenges to show the highest level of current scientific speculation. It is expected that this information will contribute to promotion of scientific understanding of quality and safety assurance of not only JP listed products but also the other biotechnological/biological products and to promotion of active discussion of each Official Monograph in JP.

1. Fundamental measures to ensure viral safety of JP listed biotechnological/biological products

The biotechnological/biological product JP includes the products derived from living tissue and body fluid (urine, blood, etc.) of mammals, etc. In near future, protein drugs derived from cell lines of human or animal origin (e.g., recombinant DNA drug, cell culture drug) will be included. The fundamental measures required for comprehensive viral safety of JP listed biotechnological/biological products are as follows: 1) acquaintance of possible virus contamination (source of contamination); 2) careful examination of eligibility of raw materials and their sources, e.g. human/animal, and thorough analysis and screening of the sample chosen as a substrate for drug production (e.g., pooled body fluid, cell bank, etc.) to determine any virus contamination and determination of type and nature of the virus, if contaminated; 3) evaluation to determine virus titer and virus-like particles hazardous to human, if exists; 4) selection of production related material (e.g., reagent, immune antibody column) free from infectious or pathogenic virus; 5) performance of virus free test at an appropriate stage of manufacturing including the final product, if necessary; 6) adoption of effective viral clearance method in the manufacturing process to remove/inactivate virus. Combined method sometimes achieves higher level of clearance; 7) development of a deliberate viral clearance scheme; 8) performance of the test to evaluate viral removal and inactivation. It is considered that the stepwise and supplemental adoption of the said measures will contribute to ensure viral safety and its improvement.

2. Safety assurance measures described in the Official Monograph and this General Information

As mentioned in above 1, this General Information describes, in package, points to be concerned with and concrete information on the measures taken for viral safety of JP listed products. Except where any specific caution is provided in Official Monograph of a product in question, Official Monograph provides in general that ''Any raw material, substrate for drug production and production related material used for production of drug should be derived from healthy animals and should be shown to be free of latent virus which is infectious or pathogenic to human'', ''Cell line and culture method well evaluated in aspects of appropriateness and rationality on viral safety are used for production, and the presence of infectious or pathogenic latent virus to human in process related materials derived from living organisms should be denied''. and ''biotechnological/biological drug should be produced through a manufacturing process which is capable of removing infectious or pathogenic virus'', etc., to raise awareness on viral safety and on necessity to conduct test and process evaluation for viral safety.

3. Items and contents described in this General Information

As for viral safety of protein drug derived from cell line of human or animal origin, there is a Notice in Japan entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare) to reflect the internationally harmonized ICH Guideline, and as for blood plasma protein fraction preparations, there is a document entitled ''Guideline for ensuring viral safety of blood plasma protein fraction preparations''. This General Information for ensuring viral safety of JP listed biotechnological/biological products has been written, referencing the contents of those guidelines, to cover general points and their details to be concerned for ensuring viral safety of not only JP listed biotechnological/biological products but also all products which would be listed in JP in future, i.e., biological products derived from living tissue and body fluid, such as urine, and protein drugs derived from cell line of human or animal origin (Table 1).

Table 1. Items described in General Information for Viral Safety Assurance of JP listed Biotechnological/Biological Product

I. Introduction
1. Fundamental measures to ensure viral safety of JP listed biotechnological/biological products
2. Safety assurance measures described in the Official Monograph and this General Information
3. Items and contents described in this General Information

II. General Matters
1. Purpose
2. Background
3. Unknown risk on the measures taken for ensuring viral safety
4. Applicable range
5. Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)
6. Basis for ensuring viral safety
7. Limit of virus test
8. Roles of viral clearance studies

III. Raw material/substrate for drug production
1. Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto
2. Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

IV. Points of concern with respect to manufacturing and virus testing
1. Virus test conducted in advance of purification process
2. Virus test as an acceptance test of an intermediate material, etc.
3. Virus test on a final product

V. Process evaluation on viral clearance
1. Rationale, objective and general items to be concerned with respect to viral clearance process evaluation
2. Selection of virus
3. Design of viral clearance studies
4. Interpretation of viral clearance studies
   1) Evaluation of viral clearance factor
   2) Calculation of viral clearance index
   3) Interpretation of results and items to be concerned at evaluation

VI. Statistics
1. Statistical considerations for assessing virus assays
2. Reproducibility and confidence limit of viral clearance studies

VII. Re-evaluation of viral clearance

VIII. Measurement for viral clearance studies
1. Measurement of virus infective titer
2. Testing by nucleic-acid amplification test (NAT)

IX. Reporting and preservation

X. Others

3.1 Purpose

The purpose of this document is to propose the comprehensive concepts of the measures to be taken for ensuring viral safety of biotechnological/biological products derived from living tissue or body fluid of mammals, etc. and of protein drugs derived from cell lines of human or animal origin. That is to say, this document describes the measures and the points of concern on the items, such as ① consideration of the source of virus contamination; ② appropriate evaluation on eligibility at selecting the raw material and on qualification of its source, e.g. human or animal; ③ virus test, and its analysis and evaluation at a stage of cell substrate for drug production; ④ appropriate evaluation to choose product related materials derived from living organisms (e.g. reagent, immune antibody column, etc.); ⑤ conduct of necessary virus test on the product at an appropriate stage of manufacturing; ⑥ development of viral clearance test scheme; ⑦ performance and evaluation of viral clearance test. This document is also purposed to comprehensively describe in details that supplemental and combining adoption of the said measures will contribute to secure viral safety and its improvement.

3.2 Background

One of the most important issues to be cautioned for safety of a biological product, which is directly derived from human or animal, or of a protein drug, which is derived from cell line of human or animal origin (recombinant DNA derived product, cell culture derived product, etc.), is risk of virus contamination. Virus contamination may cause serious situation at clinical use once it occurs. Virus contamination may be from a raw material or from a cell substrate for drug production, or may be from an adventitious factor introduced to the manufacturing process.

JP listed biological drugs or protein drugs derived from cell line have achieved drastic contribution to the medical society, and to date, there has not been any evidence of any safety problem on them caused by virus. But, social requirement of health hazard prevention is strong, and it is now

very important to prevent accidental incidence, taking security measures carefully supported by scientific rationality. It is always great concern among the persons involved that under what sort of viewpoint and to what extent we have to pursue for ensuring viral safety of a biotechnological /biological product. Before discussing these issues, two fundamental points have to be reconfirmed. One is that; we have to consider scientific, medical, and social profiles a drug has. In other words, ''Medicine is a social asset which is utilized in medical practice paying attention to the risk and benefit from the standpoints of science and society''. It is the destine and the mission of the medical/pharmaceutical society to realize prompt and stable supply of such a social asset, drug, among the medical work front to bring gospel to the patients.

The other is that; issue of viral safety is independent from safety of the components of a drug per se (narrow sense of safety). It is important to consider that this is the matter of general safety of drug (broad sense of safety). In case of a drug which has been used for a long time in the medical front, such as a JP listed product, its broad sense of safety is considered to have been established epidemiologically, and its usage past records have a great meaning. However, different from safety of drug per se (its components), taking into account any possibility of virus contamination, we have to say that only the results accumulated can not always assure viral safety of a drug used in future. Accordingly, the basis for securing broad sense of viral safety of JP listed biotechnological/biological products is to pay every attention to the measures to take for prevention, while evaluating the accumulated results.

Adopting strict regulations and conducting tests at maximum level to the extent theoretically considered may be the ways off assuring safety, but applying such way generally, without sufficient scientific review of the ways and evaluation of usage results, causes excessive requirement of regulation and test not having scientific rationality. As the results, effective and prompt supply of an important drug, already having enough accumulation of experiences, to the medical work front will be hampered, and the drug, a social asset, may not to be utilized effectively. Medicine is a sword used in medical field having double-edge named effectiveness and safety. Effectiveness and safety factors have to be derived as the fruits of leading edge of science, and relatively evaluated on a balance sheet of usefulness. Usefulness evaluation should not be unbalanced in a way that too much emphasis is placed on safety concern without back-up of appropriate scientific rationality. A drug can play an important role as a social asset only when well balanced appropriate scientific usefulness evaluation in addition to social concern of the age are given. In other words, drug is a common asset utilized by society for medication as a fruit of science of the age, and the key point of its utilization lies on a balance of risk and benefit produced from scientific and social evaluation. So, those factors have to be taken into account when target and pursuance levels for ensuring viral safety of a JP listed biotechnological/biological product are reviewed.

And, in general, the risk and benefit of drugs should be considered with the relative comparison to alternative drugs or medical treatment. The usefulness of certain drug should be reviewed finally after the competitive assessment on the risk and benefit on the alternative drugs, relevant drugs and/or alternative medical treatment.

Under such background, the purpose of this article is to describe the scientific and rational measures to be taken for ensuring viral safety of JP listed biotechnological/biological products. Giving scientific and rational measures mean that; appropriate and effective measures, elaborated from the current scientific level, are given to the issues assumable under the current scientific knowledge. In other words, possible contaminant virus is assumed to have the natures of genus, morph, particle size, physical/chemical properties, etc. which are within the range of knowledge of existing virology, and is those assumed to exist in human and animal, tissue and body fluid, which are the source of biotechnological /biological product, reagent, material, additives, etc. Accordingly, viral clearance studies using a detection method which target those viruses have to be designed.

3.3 Unknown risk on the measures taken for ensuring viral safety

There are known and unknown risks.

It is easy to determine a test method and an evaluation standard on the known risk, which exists in the drug per se (pharmaceutical component) or inevitably exists due to a quality threshold, and quantification of such risk is possible. In other words, it is easy to evaluate the known risk on a balance sheet in relation to the benefit, and we can say that valuation even in this respect has been established to some extent.

On the other hand, as for the unknown risk which is inevitable for ensuring viral safety, the subject of the risk can not be defined and quantitative concept is hard to introduce, and, therefore, taking a counter measure and evaluating its effect are not so easy. Therefore, this is the subject to be challenged calling upon wisdom of the related parties among the society of drug.

Talking about the unknown risk, there are view points that say ''It is risky because it is unknown.'' and ''What are the unknowns, and how do we cope with them in ensuring safety?''.

The view of ''It is risky because it is unknown.'' is already nothing but a sort of evaluation result, and directly connects to a final decision if it can be used as a drug. Such evaluation /decision has to be made based upon a rational, scientific or social judgment.

For example, in the case that ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was detected, but its identification could not be confirmed, and, therefore, its risk can not be denied.'', the evaluation of ''It is risky because it is unknown.'' is scientifically rational and reasonable. On the other hand, however, if we reach a decision of ''It is risky because it is unknown.'' due to the reason that ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a 'concern' that something unknown may exist.'', it can not be said that such evaluation is based upon a rational, scientific or social

judgment. It goes without saying that the utmost care has to be taken for viral safety, but the substance of 'concern' has to be at least clearly explainable. Otherwise, the 'concern' may result in causing contradiction in the meaningful mission to utilize a social asset, drug, in medical practice.

From scientific view point, we should not be narrow minded by saying ''it is risky'' because ''there is a 'concern' that something unknown may exists'', but challenge to clarify the subject of ''What is unknown, and how to cope with it for ensuring safety'' using wisdom. What is important at the time is to define ''what is unknown'' based upon current scientific knowledge. Only through this way, is it possible for us to elaborate the measures for ensuring safety.

Once we chase up the substance of unknown risk for viral safety without premises of ''what is unknown'', ''unknown'' will be an endless question because it theoretically remains unresolved forever. If this kind of approach is taken, the issue and the measure can not be scientifically connected to each other, which will result in the excessive requirement of regulation and of test to be conducted. Yet, it is unlikely that the measure which has no relation with science will be effective to the subject of ''What is unknown is unknown.''

For example, ''what is unknown'' at the ''evaluation of a purification process which can completely clear up every virus that contaminated in a manufacturing process'' should be the subject of ''what sort of existing virus that contaminated is unknown'', not on the subject of ''what sort of virus that exist in the world is unknown. In the former subject, the premise of the study is based on all the knowledge on viruses including DNA/RNA-virus, virus with/without envelope, particle size, physical/chemical properties, etc. The premise is that the virus contaminated should be within range of existing wisdom and knowledge of virus such as species, type, nature, etc., even though the virus that contaminated is unknown. Under such premise, when evaluation is made on a purification process to decide its capability of clearing a derived virus, which is within the range of existing wisdom and learning, specific viral clearance studies designed to combine a few model viruses with different natures, such as type of nucleic acid, with/without envelope, particle size, physical/chemical properties, etc., would be enough to simulate every sort of the virus already known, and will be a ''good measure for ensuring safety''.

The issue of ''the sort of viruses that exist in the world is unknown'' may be a future study item, but it is not an appropriate subject for the viral clearance test. Further, even if the subject of ''unknown viruses, which have a particle size smaller than that of currently known viruses, may exists'' or ''unknown viruses, which have special physical/chemical properties that can not be matched to any of the currently known viruses, may exists'' is set up as an armchair theory, any experimental work can not be pursued under the current scientific level, since such virus model is not available. Further, any viral clearance test performed by using the currently available methods and technologies will be meaningless ''for ensuring safety'', since particle size or natures of such speculated virus are unknown. Likewise, any counter measures can not be taken on the subject of ''unknown virus, which can not be detected by currently available screening method, may exist'', and conducting any virus detection test at any stage will be useless ''for ensuring safety''.

The requirement of regulations or tests excessively over scientific rationality will raise human, economical and temporal burden to the pharmaceutical companies, and will adversely affect prompt, effective and economical supply of a drug to the medical front. As drug is a sort of social asset, which has to be scientifically evaluated, how to assure maximization of its safety by means of scientifically rational approach at minimum human, economical and time resources is important.

It is also important to reconfirm that achievement of those issues is on the premise that appropriate measures are taken on the supply source of drugs. For example, in a case of ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a 'concern' that something unknown may exist.'', appropriateness of the test, which resulted in the judgment that ''virus, virus-like particle or retrovirus was not detected in a process of drug production'', should be a prerequisite premise when judged by science standard ate the time. If there is any question on the premise, it is quite natural that the question of ''there is a 'concern' that unknown something may exist.'' will be effective.

3.4 Applicable range

This General Information is on JP listed biological products, derived from living tissue or body fluid, and protein drugs, derived from human or animal cell line, that in Japan. In the case of protein drugs derived from human or animal cell line, the products developed and approved before enforcement of the Notice Iyakushin No. 329 entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' should have been treated under the Notice had there been one, and it is inevitable that some products approved after the Notice might not have been sufficiently treated. It is expected that such biodrug will be sufficiently examined to meet such General Information before being listed in JP. On the other hand, blood preparations listed in the biological products standard and covered by ''Guideline for securing safety of blood plasma protein fraction preparations against virus'', are out of the scope of this General Information. Further, in case of a relatively lower molecular biogenous substance, such as amino acid, saccharide and glycerin, and of gelatin, which is even classified as infectious or pathogenic polymer, there are cases that viral contamination can not be considered due to its manufacturing or purification process, and that potent viral inactivation/removal procedure that can not be applied to protein, can be used, and, therefore, it is considered reasonable to omit such substances from the subject for application. However, some part of this General Information may be used as reference. Further, a comprehensive assurance measure for viral safety is recommendable on a biotechnological/biological product not listed in JP using this document as a reference so long as it is similar to the biotechnological/biological product JP.

3.5 Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)

Promoting awareness of virus contamination to a JP listed biotechnological/biological product (source of virus contamination) and citing countermeasure thereof are important for eradicating any possible virus contamination and raising probability of safety assurance. Many biotechnological/biological products are produced from a ''substrate'' which is derived from human or animal tissue, body fluid, etc. as an origin/raw material, and in purification or pharmaceutical processing of such products column materials or additives, which are living organism origin, are occasionally used. Accordingly, enough safety measures should be taken against diffusion of the contaminant virus. Further, as mentioned in Notice Iyakushin No. 329, any protein drug derived from cell lines of human or animal origin should be carefully examined with respect to the risk of virus contamination through the cell line, the cell substrate for drug production, and through the manufacturing process applied thereafter.

''Substrate for drug production'' is defined as a starting material which is at a stage where it is deemed to be in a position to ensure quality/safety of an active substance. The ''substrate for drug production'' is sometimes tissue, body fluid, etc. of human or animal per se and pooled material such as urine, and sometimes a material after some treatment. In many cases, it is considered rational that starting point of full-scale test, evaluation and control should be at the stage of ''substrate for drug production''. The more strict levels of test, evaluation and control achieved at the stage of ''substrate for drug production'' can more rationalize evaluation and control of the raw material or individual level of upper stream. On the contrary, strict evaluation and control of the raw material or individual level at an upper stream stage can rationalize tests, evaluation or quality control at the stage of ''substrate for drug production''.

The measures taken for ensuring viral safety on a biotechnological/biological product currently listed in JP can be assumed from the provisions of manufacturing method, specification and test methods of each preparation. However, unitary principles or information with respect to the measures to be taken for ensuring viral safety, totally reviewing the entire process up to the final product rationally and comprehensively, including source/raw material/substrate, purification process, etc. have not been clarified. The most important thing for ensuring viral safety is to take thorough measures to eliminate the risk of virus contamination at any stage of source animal, raw material and substrate. Although not the cases of a biotechnological/biological product, known examples of a virus contamination from a raw material/substrate for drug production in old times are Hepatitis A Virus (HAV) or Hepatitis C Virus (HCV) contamination in blood protein fraction preparations. It is also well known that Human Immunodeficiency Virus (HIV) infection caused by blood plasma protein fraction preparations occurred in 1980s. The aim of this General Information is to show concrete guidelines for comprehensive viral safety assurance of the JP listed biotechnological/biological products. The pathogenic infectious viruses, currently known to contaminate to raw materials, etc. of drug and have to be cautioned, are HIV, HAV, Hepatitis B Virus (HBV), HCV, Human T-Lymphotropic Virus (HTLV-I/II), Human Parvovirus B19, Cytomegalovirus (CMV), etc. Biotechnological/biological products produced from raw material/cell substrate derived from tissue or body fluid of human or animal origin always have a risk of contamination of pathogenic or other latent virus. Therefore, safety measures should be thoroughly taken. There is also the case that a material, other than the biological component such as raw material/substrate, causes virus contamination. Using enzymatic or monoclonal antibody column or using albumin etc. as a stabilizer, is the example of the case, in which caution has to be taken on risk of virus contamination from the source animal or cell. Further, there is a possibility of contamination from environment or personnel in charge of production or at handling of the product. So, caution has to be taken on these respects as well.

In case of protein drugs derived from cell line of human or animal origin, there may be cases where latent or persistent infectious viruses (e.g., herpesvirus) or endogenous retroviruses exist in the cell. Further, adventitious viruses may be introduced through the routes such as: 1) derivation of a cell line from an infected animal; 2) use of a virus to drive a cell line; 3) use of a contaminated biological reagent (e.g., animal serum components); 4) contamination during cell handling. In the manufacturing process of drug, an adventitious virus may contaminate to the final product through the routes, such as 1) contamination through a reagent of living being origin, such as serum component, which is used for culturing, etc.; 2) use of a virus for introduction of a specific gene expression to code an objective protein; 3) contamination through a reagent used for purification such as monoclonal antibody affinity column; 4) contamination through an additive used for formulation production; 5) contamination at handling of cell and culture media, etc. It is reported that monitoring of cell culture parameter may be helpful for early detection of an adventitious viral contamination.

3.6 Basis for ensuring viral safety

Viral safety of a biotechnological/biological product produced from a raw material/substrate, which derived from tissue, body fluid, cell line, etc. of human or animal origin, can be achieved by supplemental and appropriate adoption of the following plural methods.

(1) Acquaintance of possible virus contamination (source of contamination).
(2) Careful examination of eligibility of the raw material and its source, i.e., human or animal, thorough analysis and screening of the sample chosen as the substrate for drug production to determine virus contamination and through examination of the type of virus and its nature, if contaminated.
(3) Evaluation to determine hazardous properties of the virus or virus-like particle to human, if exists.
(4) Choosing a product related material of living organism origin (e.g., reagent, immune anti-body column, etc.)

which is free from infectious or pathogenic virus.

(5) Conduct virus free test at an appropriate stage of manufacturing including the final product, if necessary.

(6) Adoption of an effective method to remove/inactivate the virus in the manufacturing process for viral clearance. Combined processes sometimes achieve higher level of viral clearance.

(7) Develop a deliberate viral clearance scheme.

(8) Conduct test and evaluation to confirm removal/inactivation of the virus.

Manufacturer is responsible for explaining rationality of the way of approach adopted among the comprehensive strategy for viral safety on each product and its manufacturing process. At the time, the approach described in this General Information shall be applicable as far as possible.

3.7 Limit of virus test

Virus test has to be conducted to define existence of virus, but it should be noted that virus test alone can not reach a conclusion of inexistence of virus nor sufficient to secure safety of the product. Examples of a virus not being detected are as follows: 1) Due to statistical reason, there is an inherent quantitative limit, such as detection sensitivity at lower concentration depends upon the sample size. 2) Generally, every virus test has a detection limit, and any negative result of a virus test can not completely deny existence of a virus. 3) A virus test applied is not always appropriate in terms of specificity or sensitivity for detection of a virus which exists in the tissue or body fluid of human or animal origin.

Virus testing method is improved as science and technology progress, and it is important to apply scientifically the most advanced technology at the time of testing so that it can be possible to raise the assurance level of virus detection. It should be noted, however, that the limit as mentioned above can not always be completely overcome. Further, risk of virus contamination in a manufacturing process can not be completely denied, and, therefore, it is necessary to elaborate the countermeasure taken these effects into account.

Reliable assurance of viral free final product can not be obtained only by negative test results on the raw material/substrate for drug production or on the product in general, it is also necessary to demonstrate inactivation/removal capability of the purification process.

3.8 Roles of viral clearance studies

Under the premises as mentioned in the preceding clause that there is a limit of a virus test, that there is a possibility of existence of latent virus in a raw material/substrate for drug production and that there is a risk of entry of a non-endogenous virus in a manufacturing process, one of the important measures for viral safety is how to remove or inactivate the virus, which exists in a raw material, etc. and can not be detected, or the virus, which is contingently contaminated in a manufacturing process. The purpose of viral clearance study is to experimentally evaluate the viral removal/inactivation capability of a step that mounted in a manufacturing process. So, it is necessary to conduct an experimental scale spike test using an appropriate virus that is selected by taking account the properties, such as particle size, shape, with or without envelope, type of nucleic acid (DNA type, RNA type), heat and chemical treatment tolerance, etc., with an aim to determine removal/inactivation capability of the virus that can not be detected in a raw material or contingently contaminated.

As mentioned above, the role of viral clearance study is to speculate removal/inactivation capability of a process through a model test, and it contributes to give scientific basis to assure that a biotechnological/biological product of human or animal origin has reached an acceptable level in aspect of viral safety.

At a viral clearance study, it is necessary to adopt an appropriate approach method which is definitive and rational and can assure viral safety of a final product, taking into consideration the source and the properties of the raw material/substrate as well as the manufacturing process.

4. Raw material/substrate for drug production

4.1 Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto

For manufacturing JP listed biotechnological/biological products, which require measures for viral safety, a raw material/substrate derived mainly from human, bovine, swine or equine is used, and it is obvious that such human and animal has to be healthy nature. A wild animal should be avoided, and it is recommended to use animals derived from a colony controlled by an appropriate SPF (Specific Pathogen-Free) condition and bred under a well deigned hygienic control, including appropriate control for prevention of microbial contamination and contamination monitoring system. If a meat standard for food is available, an animal meeting this standard has to be used. The type of virus to be concerned about depend on animal species, but it may be possible to narrow down the virus for investigation by means of examining the hygiene control, applicability of a meat standard for food, etc. On the other hand, even with the animals of the same species, a different approach may be necessary depending upon the region where the specimen for a raw material/substrate is taken. For example, in case of obtaining raw material/substrate from blood or other specific region, it is necessary to be aware of the risk level, virus multiplication risk, etc. which may specifically exists depending upon its region. Such approach may be different from those applied to body waste such as urine, milk, etc. as a source of raw material/substrate. Further, caution has to be taken on transmissible spongiform encephalopathy (TSE) when pituitary gland, etc. is used as a raw material. This report does not include detailed explanation on TSE, but recommendations are to use raw material derived from 1) animals originated in the countries (area) where incidence of TSE has not been reported; 2) animals not infected by TSE; or 3) species of animal which has not been reported on TSE. It is recommended to discuss the matters concerned with TSE with the regulatory authority if there is any unclear point.

Followings are the raw material/substrate used for manufacturing biotechnological/biological products in

Japan.

(1) Biological products derived from human

Blood plasma, placenta, urine, etc. derived from human are used as the sources of raw material of biotechnological/biological product. As for these raw materials, there are 2 cases: 1) Appropriateness can be confirmed by interview or by examination of the individual who supplies each raw material, and 2) Such sufficient interview or examination of the individual can not be made due to type of raw material. In case that sufficient examination of individual level is not possible, it is necessary to perform test to deny virus contamination at an appropriate manufacturing stage, for example, the stage to decide it as a substrate for drug production.

(2) Biological products derived from animal besides human

Insulin, gonadotropin, etc. are manufactured from blood plasma or from various organs of bovine, swine and equine.

(3) Protein drug derived from cell line of human or animal origin

It is expected that protein drugs derived from cell line of human or animal origin will be listed in JP in a near future. In the case of these products, a cell line of human or animal is the raw material per se, and the substrate for drug production is a cell bank prepared from cloned cell line (master cell bank or working cell bank). Examination at cell bank level is considered enough for viral safety qualification, but it goes without saying that the more appropriate and rational qualification evaluation test of cell bank can be realized when more information is available on the virus of the source animal or on prehistory of driving the cell line, the base of cell bank.

4.2 Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

(1) Biological products derived from human

Body fluid etc. obtained from healthy human must be used for biological products production. Further, in case that interview or examination of the individual, who supplies the raw material, can be possible and is necessary, interview under an appropriate protocol and a serologic test well evaluated in aspects of specificity, sensitivity and accuracy have to be performed, so that only the raw material, which is denied latent HBV, HCV and HIV, will be used. In addition to the above, it is necessary to test for gene of HBV, HCV and HIV by a nucleic amplification test (NAT) well evaluated in aspects of specificity, sensitivity and accuracy.

In case of the raw material (e.g., urine), which can not be tested over the general medical examination of the individual who supplies the material, or of the raw material which is irrational to conduct individual test, the pooled raw material, as the substrate for drug production, has to be conducted at least to deny existence of HBV, HCV and HIV, using a method well evaluated in aspects of specificity, sensitivity and accuracy, such as the antigen test or NAT.

(2) Biological products derived from animal besides human

The animal used for manufacturing biological product has to be under appropriate health control, and has to be confirmed of its health by various tests. Further, it is necessary that the population, to which the animal belongs, has been under an appropriate breeding condition, and that no abnormal individual has been observed in the population. Further, it is necessary to demonstrate information or scientific basis which can deny known causes infection or disease to human, or to deny such animal inherent latent virus by serologic test or by nucleic amplification test (NAT). The infectious virus that is known to be common between human and animal, and known to cause infection in each animal are tentatively listed in Table 2. It is necessary that the table is completed under careful examination, and denial of all of them, by means of tests on individual animal, tissue, body fluid, etc. as a raw material, or on pooled raw material (as a direct substrate for drug production), is not always necessary. Table 2 can be used as reference information, in addition to the other information, such as; source of animal, health condition, health and breeding control, conformity to the meat standard for food, etc., to elaborate to which virus what kind of test has to be performed, and for which virus it is not always necessary to test for, etc. It is important to clarify and record the basis of choosing the virus and the test conducted thereof.

Table 2. Infectious viruses known to be common between human and animal and known to cause infection to each animal

	bovine	swine	sheep	goat	equine
Cowpox virus	◎				
Paravaccinia virus	◎	◎	◎	◎	
Murry valley encephalitis virus	◎	◎			
Louping-ill virus	◎	◎	◎	◎	
Wesselsbron virus			◎		
Foot-and-mouth disease virus	◎	◎			
Japanese encephalitis virus		◎			
Vesicular stomatitis virus		◎			
Bovine papular stomatitis virus	◎				
Orf virus			◎		
Borna disease virus			◎		◎
Rabies virus	◎	◎	◎	◎	◎
Influenza virus		◎			
Porcine hepatitis E virus		◎			
Encephalomyocarditis virus	◎	◎			
Rota virus	◎				

Eastern equine encephalitis virus					◎
Western equine encephalitis virus					◎
Venezuelan equine encephalitis virus					◎
Morbillivirus					◎
Hendravirus					◎
Nipahvirus		◎			
Transmissible gastroenteritis virus		◎			
Porcine respiratory coronavirus		◎			
Porcine epidemic diarrheavirus		◎			
Hemagglutinating encephalomyelitis virus		◎			
Porcine respiratory and reproductive complex virus		◎			
Hog cholera virus		◎			
Parainfluenza 3 virus		◎			
Telfan/Teschen disease virus		◎			
Reoviruses		◎			
Endogenous retrovirus		◎			
Porcine adenovirus		◎			
Porcine circovirus		◎			
Porcine parvovirus		◎			
Swine poxvirus		◎			
Porcine cytomegalovirus		◎			
Pseudorabies virus		◎			
Russian spring summer encephalitis virus			◎	◎	
Rift Valley fever virus			◎	◎	
Crimean-Congo hemorrhagic fever virus (Nairovirus)	◎		◎	◎	
Torovirus	◎				

(3) Protein drug derived from cell line of human or animal origin

It is important to conduct thorough investigation on latent endogenous and non-endogenous virus contamination in a master cell bank (MCB), which is the cell substrate for drug production, in accordance with the Notice Iyakushin No. 329 entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin''. Further, it is necessary to conduct an appropriate adventitious virus test (e.g., *in vitro* and *in vivo* test) and a latent endogenous virus test on the cell at the limit of *in vitro* cell age (CAL) for drug production. Each WCB as a starting cell substrate for drug production should be tested for adventitious virus either by direct testing or by analysis of cells at the CAL, initiated from the WCB. When appropriate non-endogenous virus tests have been performed on the MCB and cells cultured up to or beyond the CAL have been derived from the WCB and used for testing for the presence of adventitious viruses, similar tests need not be performed on the initial WCB.

5. Points of concern with respect to manufacturing and virus testing

To ensure viral safety of a biological product derived from tissue, body fluid etc. of human or animal origin, it is necessary to exclude any possibility of virus contamination from a raw material, such as tissue and body fluid, or a substrate, paying attention to the source of virus contamination as mentioned in above 3.5, and to adopt appropriate manufacturing conditions and technologies in addition to enhancement of manufacturing environment, so that virus contamination in the course of process and handling and from operators, facilities and environment can be minimized.

In addition to the above, effective virus test and viral inactivation/removal technology, which are reflected by rapid progress of science, have to be introduced. Adoption of two or more steps with different principles is recommended for virus inactivation/removal process. Further, it is important to minimize any possible virus derivation by using a reagent, which quality is equivalent to that of a drug. Examples of virus inactivation/removal processes are ① heating (It is reported that almost viruses are inactivated by heating at 55 – 60°C for 30 minutes with exceptions of hepatitis virus, etc. and that dry heating at 60°C for 10 – 24 hours is effective in case of the products of blood or urine origin.), ② treatment with organic solvent/surfactant (S/D treatment), ③ membrane filtration (15 – 50 nm), ④ acid treatment, ⑤ irradiation (γ-irradiation, etc.), ⑥ treatment with column chromatograph (e.g. affinity chromatography, ion-exchange chromatography), ⑦ fractionation (e.g. organic solvent or ammonium sulfate fractionation), ⑧ extraction.

5.1 Virus test conducted in advance of purification process

(1) Biological products derived from human

In many cases, samples for virus test before purification process are body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. As mentioned in 4.2 (1), it is necessary to deny latent HBV, HCV and HIV by the test evaluated enough in aspects of specificity, sensitivity and accuracy. Even in a case that a non-purified bulk before purification process is produced from a substrate, it is not always necessary to conduct virus test again at the stage before purification, so long as the presence of any latent virus can be denied at the stage of substrate by an appropriate virus test, with cases where the non-purified bulk is made from the substrate by adding any reagent etc. of living organisms origin are an exception.

(2) Biological products derived from animal besides human

Similar to (1) above, samples for virus test before purification process are, in many cases, body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. In these cases, it is necessary to have a data, which can deny latent virus of probable cause of human infection or disease as mentioned in the above 4.2 (2), or to have a result of serologic test or nucleic amplification test (NAT) evaluated enough in aspects of specificity, sensitivity and accuracy. The concept, which is applied to a case that non-purified bulk before purification process is produced from substrate, is the same as those provided in the above 4.2 (1).

(3) Protein drug derived from cell line of human or animal origin

Generally, substrate in this case is cell bank, and the sample for testing before purification process is a harvested cell after cell culturing or unprocessed bulk which consists of single or pooled complex culture broth. The unprocessed bulk may be sometimes culture broth without cell. Denial of latent virus, which is determined by virus test at a MCB or WCB level, does not always deny latent virus in unprocessed bulk after culturing. Further, it is noted that the viral test at the CAL is meaningful as a validation but can not guarantee definite assurance of latent virus denial, since the test is generally performed only once. In case of using a serum or a component of blood origin in a culture medium, definite denial of latent virus at the level of unprocessed bulk can not be assured so long as the viral test has not been conducted on each lot at the CAL, since lot renewal can be a variable factor on viral contamination.

A representative sample of the unprocessed bulk, removed from the production reactor prior to further processing, represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic). In certain instances it may be more appropriate to test a mixture consisting of both intact and disrupted cells and their cell culture supernatants removed from the production reactor prior to further processing.

In case of unprocessed bulk, it is required to conduct virus test on at least 3 lots obtained from pilot scale or commercial scale production. It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material sources and results of viral clearance studies. Screening *in vitro* tests, using one or several cell lines, are generally employed to test unprocessed bulk. If appropriate, a NAT test or other suitable methods may be used.

Generally, harvest material in which adventitious virus has been detected should not be used to manufacture the product. If any adventitious viruses are detected at this level, the process should be carefully checked to determine the cause of the contamination, and appropriate actions taken.

5.2 Virus test as an acceptance test of an intermediate material, etc.

When a biological product is manufactured from tissue, body fluid etc. of human or animal origin, there are cases that an intermediate material, partially processed as a raw material or substrate by outside manufacturer, is purchased and used for manufacturing. In such case, if any test to meet this General Information has been conducted by such outside manufacturer, it is necessary for the manufacturer of the biological product, who purchased the intermediate material, to examine what sort of virus test has to be conducted as acceptance tests, and to keep record on the basis of rationality including the details of the test conducted.

On the other hand, if no test to meet this General Information has been conducted by such outside manufacturer of the raw material, all necessary virus free test has to be conducted to meet this General Information on the intermediate material regarding it as the direct substrate for drug production.

5.3 Virus test on a final product

Virus tests to be conducted on a final product (or on a product to reach the final product) has to be defined under comprehensive consideration of the type of raw material or substrate, the result of virus test conducted on raw material/substrate, the result of evaluation on viral removal/inactivation process, any possibility of virus contamination in the manufacturing process, etc. Comprehensive viral safety assurance can only be achieved by appropriate selection of the raw material/substrate, an appropriate virus test conducted on the raw material/substrate/intermediate material, the virus test conducted at an appropriate stage of manufacturing, an appropriate viral clearance test, etc. However, there are cases of having specific backgrounds, such as 1) use of the raw material derived from unspecified individual human, 2) possible existence of virus at window period, 3) specific detection limit of virus test, etc. and in these cases, virus contamination to the final product may occur if there is any deficiency on the manufacturing process (e.g., damage of membrane filter) or any mix-up of the raw materials, etc. To avoid such accidental virus contamination, it may be recommended to conduct nucleic amplification test (NAT) on the final product focusing on the most risky virus among those that may possibly to exist in the raw material.

6. Process evaluation on viral clearance

6.1 Rationale, objective and general items to be concerned with respect to viral clearance process evaluation

Evaluation of a viral inactivation/removal process is important for ensuring safety of a biological product derived from tissue or body fluid of human or animal origin. Conducting evaluation on viral clearance is to assure, even to

Table 3. Example of viruses which have been used for viral clearance studies

Virus	Family	Genus	Natural host	Genome	Env	Size (nm)	Shape	Resistance
Vesicular Stomatitis Virus	Rhabd	Vesiculovirus	Equine Bovine	RNA	yes	70 × 150	Bullet	Low
Parainfluenza Virus	Paramyxo	Paramyxovirus	Various	RNA	yes	100 – 200 +	Pleo-Spher	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80 – 110	Spherical	Low
Sindbis Virus	Toga	Alphavirus	Human	RNA	yes	60 – 70	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50 – 70	Pleo-Spher	Low
Pseudorabies Virus	Helpes		Swine	DNA	yes	120 – 200	Spherical	Med
Poliovirus Sabin Type 1	Picorna	Enterovirus	Human	RNA	no	25 – 30	Icosahedral	Med
Encephalomyocarditis Virus	Picorna	Cardiovirus	Mouse	RNA	no	25 – 30	Icosahedral	Med
Reovirus 3	Reo	Orthoreovirus	Various kind	RNA	no	60 – 80	Spherical	Med
SV 40	Papova	Polyomavirus	Monkey	DNA	no	40 – 50	Icosahedral	Very high
Parvovirus: canine, porcine	Parvo	Parvovirus	Canine Porcine	DNA	no	18 – 24	Icosahedral	Very high

some extent, elimination of the virus, which may exist in a raw material, etc. or may be derived to the process due to unexpected situation. Viral clearance studies should be made by a carefully designed appropriate method, and has to be rationally evaluated.

The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition (''spiking'') of significant amounts of a virus at different manufacturing/purification steps and demonstrating its removal or inactivation during the subsequent steps. It is not necessary to evaluate or characterize every step of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps. It should be borne in mind that other steps in the process may have an indirect effect on the viral inactivation/removal achieved. Manufacturers should explain and justify the approach used in studies for evaluating viral clearance.

The reduction of virus infectivity may be achieved by removal of virus particles or by inactivation of viral infectivity. For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. For inactivation steps, the study should be planned in such a way that samples are taken at different times and an inactivation curve constructed.

6.2 Selection of virus

To obtain broad range of information of viral inactivation /removal, it is desirable that a model virus used for viral clearance studies should be chosen from the viruses with broad range of characteristics in aspects of DNA/RNA, with or without envelope, particle size, significant resistance to physical/chemical treatment, etc. and it is necessary to combine about 3 model viruses to cover these characteristics.

At choice of a model virus, there are also the ways to choose a virus closely related to or having the same characteristics of the virus known to exist in the raw material. In such case, it is in principle recommendable to choose a virus which demonstrates a higher resistance to inactivation/removal treatment if two or more candidate viruses are available for choice. Further, a virus which can grow at a high titer is desirable for choice, although this may not always be possible. In addition to the above, choosing a virus, which will provide effective and reliable assay result at each step, is necessary, since sample condition to be tested at each step of a production process may influence the detection sensitivity. Consideration should also be given to health hazard which may pose to the personnel performing the clearance studies.

For the other items taken for consideration at choice of virus, the Notice, Iyakushin No. 329 can be used as a reference. Examples of the virus which have been used for viral clearance studies are shown in Table 3 which was derived from Iyakushin No. 329. However, the Notice, Iyakushin No. 329, is on viral safety of a product derived cell line of human or animal origin, and a more appropriate model virus has to be chosen taking into account the origin/raw material of biological products.

6.3 Design of viral clearance studies

The purpose of viral clearance studies is to quantitatively evaluate removal or inactivation capability of a process, in which a virus is intentionally spiked to a specific step of a manufacturing process.

Following are the precautions to be taken at planning viral clearance studies.

(1) Care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation thus distorting the correlation with actual production.

(2) Virus detection method gives great influence to viral clearance factor. Accordingly, it is advisable to gain detection sensitivity of the methods available in advance, and use a method with a detection sensitivity as high as possible. Quantitative infectivity assays should have adequate sensitivity and reproducibility in each manufacturing process, and should be performed with sufficient replicates to ensure adequate statistical validity of the result. Quantitative assays not associated with infectivity may be used if justified. Appropriate virus controls should be included in all infectivity assays to ensure the sensitivity of the method. Also, the statistics of sampling virus when at low concentrations (for example, number of virus is 1-1000/L) should be considered.

(3) Viral clearance studies are performed in a miniature size system that simulates the actual production process of the biotechnological/biological product used by the manufacturer. It is inappropriate to introduce any virus into a production facility because of GMP constraints. Therefore, viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process. The viral clearance studies should be performed under the basic concept of GLP.

(4) Each factor on a viral clearance study of a process, which is performed in miniature size, should reflect that of actual manufacturing as far as possible, and its rationality should be clarified. In case of chromatograph process, length of column bed, linear velocity, ratio of bed volume per velocity (in other words, contact time), buffer, type of column packing, pH, temperature, protein concentration, salt concentration and concentration of the objective product are all correspondent to those of the actual production. Further, similarity of elution profile should be achieved. For the other process, similar concept should be applied. If there is any factor which can not reflect the actual production, its effect to the result should be examined.

(5) It is desirable that two or more inactivation/removal processes of different principles are selected and examined.

(6) As for the process which is expected to inactivate/remove virus, each step should be evaluated in aspect of clearance capability, and carefully determined if it is the stage of inactivation, removal or their combination for designing the test. Generally, in viral clearance test, a virus is spiked in each step which is the object of the test, and after passing through the process in question, the reduction level of infectivity is evaluated. But, in some case, it is accepted that a high potential virus is spiked at a step of the process, and virus concentration of each succeeding step is carefully monitored. When removal of virus is made by separation or fractionation, it is desirable to investigate how the virus is separated or fractionated (mass balance).

(7) For assessment of viral inactivation, unprocessed crude material or intermediate material should be spiked with infectious virus and the reduction factor calculated. It should be recognized that virus inactivation is not a simple, first order reaction and is usually more complex, with a fast ''phase 1'' and a slow ''phase 2''. The study should, therefore, be planned in such a way that samples are taken at different times and an inactivation curve constructed. It is recommended that studies for inactivation include at least one time point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time. The reproducible clearance should be demonstrated in at least two independent studies. When there is a possibility that the virus is a human pathogen, it is very important that the effective inactivation process is designed and additional data are obtained. The initial virus load should be determined from the virus which can be detected in the spiked starting material. If this is not possible, the initial virus load may be calculated from the titer of the spiking virus preparation. Where inactivation is too rapid to plot an inactivation curve using process conditions, appropriate controls should be performed to demonstrate that infectivity is indeed lost by inactivation.

(8) If antibody against virus exists in an unprocessed material, caution should be taken at clearance studies, since it may affect the behavior of virus at viral removal or inactivation process.

(9) Virus spiked in unprocessed material should be sufficient enough to evaluate viral removal or inactivation capability of the process. However, the virus ''spike'' to be added to the unprocessed material should be as small as possible in comparison with the sample volume of the unprocessed material so as not to cause characteristic change of the material by addition of the virus nor to cause behavioral change of the protein in the material.

(10) It is desirable that the virus in the sample is subject for quantitative determination without applying ultracentrifuge, dialysis, storage, etc. as far as possible. However, there may be a case that any handling before quantitative test, such as remove procedure of inhibitor or toxic substance, storage for a period to realize test at a time, etc., is inevitable. If any manipulation, such as dilution, concentration, filtration, dialysis, storage, etc., is applied for preparation of the sample for testing, a parallel control test, which passes through a similar manipulation, should be conducted to assess infectivity variance at the manipulation.

(11) Buffers and product (desired protein or other component contained therein) should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary. If the product itself has anti-viral activity, the clearance study may need to be performed without the product in a ''mock'' run, although omitting the product or substituting a similar protein that does not have anti-viral activity could affect the behaviour of the virus in some production steps.

(12) Many purification schemes use the same or similar

buffers or columns, repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used.

(13) Overall reduction factors may be underestimated where production conditions or buffers are too cytotoxic or virucidal and should be discussed on a case-by-case basis. Overall reduction factors may also be overestimated due to inherent limitations or inadequate design of viral clearance studies.

(14) It has to be noted that clearance capability of viral removal/inactivation process may vary depending upon the type of virus. The viral removal/inactivation process, which displays viral clearance by specific principle or mechanism, may be quite effective to the virus, which meets such mechanism of action, but not effective to the other type of viruses. For example, S/D treatment is generally effective to the virus with lipid membrane, but not effective to the virus not having such membrane. Further, some virus is resistant to the general heating process (55 – 60°C, 30 minutes). When clearance is expected for such virus, introduction of a further severe condition or process, which has different principle or mechanism, is necessary. Virus removal membrane filtration, which is different from S/D or heat treatment in aspect of principle, is effective to a broad range of virus that can not pass through the membrane. Affinity chromatography process, which specifically absorbs the objective protein, can thoroughly wash out the materials other than the objective protein including virus etc. and is generally effective for viral removal. Separation/fractionation of a virus from an objective protein is sometimes very difficult, but there are not so rare that ion exchange chromatography, ethanol fractionation, etc. is effective for clearance of a virus which can not be sufficiently inactivated or removed by the other process.

(15) Effective clearance may be achieved by any of the following: multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps. Separation methods may be dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties. However, despite these potential variables, effective removal can be obtained by a combination of complementary separation steps or combinations of inactivation and separation steps. Well designed separation steps, such as chromatographic procedures, filtration steps and extractions, can be also effective virus removal steps provided that they are performed under appropriately controlled conditions.

(16) An effective virus removal step should give reproducible reduction of virus load shown by at least two independent studies.

(17) Over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary. Some estimate of the stability of the viral clearance after several uses may provide support for repeated use of such columns.

(18) The Notice, Iyakushin No. 329, would be used as a reference when viral clearance studies on biological products are designed.

6.4 Interpretation of viral clearance studies

6.4.1 Evaluation on viral clearance factor

Viral clearance factor is a logarithm of reduction ratio of viral amount (infectious titer) between each step applied for viral clearance of a manufacturing process. Total viral clearance factor throughout the process is sum of the viral clearance factor of each step appropriately evaluated.

Whether each and total viral clearance factor obtained are acceptable or should not be evaluated in aspects of every virus that can be realistically anticipated to derive into the raw material or the manufacturing process, and its rationality should be recorded.

In case that existence of any viral particle is recognized in a substrate for drug production, e.g., a substrate of rodent origin for biodrug production, it is important not only to demonstrate removal or inactivation of such virus, but also to demonstrate that the purification process has enough capability over the required level to assure safety of the final product at an appropriate level. The virus amount removed or inactivated in a manufacturing process should be compared with the virus amount assumed to exist in the substrate etc. used for manufacturing drug, and for this purpose, it is necessary to obtain the virus amount in the raw materials/substrate, etc. Such figure can be obtained by measuring infectious titer or by the other method such as transmission electron microscope (TEM). For evaluation of overall process, a virus amount, far larger than that assumed to exist in the amount of the raw materials/substrate which is equivalent to single administration of the final product, has to be removed. It is quite rare that existence of virus can be assumed in a substrate for drug production, with the exception of the substrate of rodent origin, and such suspicious raw material/substrate should not be used for manufacturing drug with a special exceptional case that the drug in question is not available from the other process and is clinically indispensable, and that the information including infectious properties of the virus particle assumed to exist has been clarified.

6.4.2 Calculation of viral clearance index

Viral clearance factor, "*R*", for viral removal/inactivation process can be calculated by the following formula.

$$R = \log[(V_1 \times T_1)/(V_2 \times T_2)]$$

In which

R: Logarithm of reduction ratio
V_1: Sample volume of the unprocessed material
T_1: Virus amount (titer) of the unprocessed material
V_2: Sample volume of the processed material
T_2: Virus amount (titer) of the processed material

At the calculation of viral clearance factor, it is recommendable to use the virus titer detected in the sample preparation of the unprocessed material after addition of virus, not the viral titer added to the sample preparation wherever possible. If this is not possible, loaded virus amount is calculated from virus titer of the solution used for spike.

6.4.3 Interpretation of results and items to be concerned at evaluation

At the interpretation and the evaluation of the data on effectiveness of viral inactivation/removal process, there are various factors to be comprehensively taken into account, such as ① appropriateness of the virus used for the test, ② design of the viral clearance studies, ③ virus reduction ratio shown in logarithm, ④ time dependence of inactivation, ⑤ factors/items which give influence to the inactivation/removal process, ⑥ sensitivity limit of virus assay method, ⑦ possible effect of the inactivation/removal process which is specific to certain class of viruses.

Additional items to be concerned at appropriate interpretation and evaluation of the viral clearance data are as follows:

(1) Behavior of virus used to the test

At interpretation of the vial clearance results, it is necessary to recognize that clearance mechanism may differ depending upon the virus used for the test. Virus used for a test is generally produced in tissue culture, but behavior of the virus prepared in the tissue culture may be different from that of the native virus. Examples are possible differences of purity and degree of aggregation between the native and the cultured viruses. Further, change of surface properties of a virus, e.g., addition of a sucrose chain which is ascribed to specific nature of a separation process, may give effect to the separation. These matters should be also considered at interpretation of the results.

(2) Design of test

Viral clearance test should have been designed taking into account variation factors of the manufacturing process and scaling down, but there still remain some variance from actual production scale. It is necessary to consider such variance at the interpretation of the data and limitation of the test.

(3) Acceptability of viral reduction data

Total viral clearance factor is expressed as a sum of logarithm of reduction ratio obtained at each step. The summation of the reduction factor of multiple steps, particularly of steps with little reduction (e.g., below 1 $\log_{10}$), may overestimate viral removal/inactivation capability of the overall process. Therefore, virus titer of the order of 1 $\log_{10}$ or less has to be ignored unless justified. Further, viral clearance factor achieved by repeated use of the same or similar method should be ignored for calculation unless justified.

(4) Time dependence of inactivation

Inactivation of virus infectivity frequently shows biphasic curve, which consists of a rapid initial phase and subsequent slow phase. It is possible that a virus not inactivated in a step may be more resistant to the subsequent step. For example, if an inactivated virus forms coagulation, it may be resistant to any chemical treatment and heating.

(5) Evaluation of viral reduction ratio shown logarithm

Viral clearance factor shown in logarithm of reduction ratio of virus titer can demonstrate drastic reduction of residual infectious virus, but there is a limit that infectious titer can never be reduced to zero. For example, reduction in infectivity of a preparation containing 8 $\log_{10}$ infectious unit per mL by a factor of 8 $\log_{10}$ leaves zero $\log_{10}$ per mL or one infectious unit per mL, taking into account the detection limit of assay.

(6) Variable factor of manufacturing process

Minor variance of a variation factor of a manufacturing process, e.g., contact time of a spiked sample to a buffer or a column, will sometimes give influence to viral removal or inactivation effect. In such case, it may be necessary to investigate to what extent such variance of the factor has given influence to the process concerned in aspect of viral inactivation.

(7) Existence of anti-viral antiserum

Anti-viral antiserum that exists in the sample preparation used for a test may affect sensitivity of distribution or inactivation of a virus, which may result in not only defusing the virus titer but complicating interpretation of the test result. So, existence of anti-viral antiserum is one of the important variable factors.

(8) Introduction of a new process for removal/inactivation

Viral clearance is an important factor for securing safety of drug. In case that an achievement level of infective clearance of a process is considered insufficient, a process which is characterized by inactivation/removal mechanism to meet the purpose or an inactivation/removal process which can mutually complement to the existence process has to be introduced.

(9) Limit of viral clearance studies

Viral clearance studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved but do not by themselves establish safety. However, a number of factors in the design and execution of viral clearance studies may lead to an incorrect estimate of the ability of the process to remove virus infectivity, as described above.

7. Statistics

The viral clearance studies should include the use of statistical analysis of the data to evaluate the results. The study results should be statistically valid to support the conclusions reached.

7.1 Statistical considerations for assessing virus assays

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays should be performed to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

Assay

1. Assay methods may be either quantal or quantitative. Both quantal and quantitative assays are amenable to statistical evaluation.

2. Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control. These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay

variation).

3. The 95% confidence limits for results of within-assay variation normally should be on the order of ± 0.5 $\log_{10}$ of the mean. Within-assay variation can be assessed by standard textbook methods. Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately 0.5 $\log_{10}$ of the mean estimate established in the laboratory for the assay to be acceptable. Assays with lower precision may be acceptable with appropriate justification.

7.2 Reproducibility and confidence limit of viral clearance studies

An effective virus inactivation/removal step should give reproducible reduction of virus load shown by at least two independent studies. The 95% confidence limits for the reduction factor observed should be calculated wherever possible in studies of viral clearance. If the 95% confidence limits for the viral assays of the starting material are $\pm s$, and for the viral assays of the material after the step are $\pm a$, the 95% confidence limits for the reduction factor are $\pm\sqrt{s^2 + a^2}$.

8. Re-evaluation of viral clearance

Whenever significant changes in the production or purification process are made, the effect of that change, both direct and indirect, on viral clearance should be considered and the system re-evaluated as needed. Changes in process steps may also change the extent of viral clearance.

9. Measurement for viral clearance studies

9.1 Measurement of virus infective titer

Assay methods may be either quantal or quantitative. Quantal methods include infectivity assays in animals or in tissue-culture-infectious-dose (TCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

9.2 Testing by nucleic-acid-amplification test (NAT)

NAT can detect individual or pooled raw material/cell substrate or virus genome at a high sensitivity even in a stage that serum test on each virus is negative. Further, it can detect HBV or HCV gene, which can not be measured in culture system. Window period can be drastically shortened at the test on HBV, HCV and HIV, and the method is expected to contribute as an effective measure for ensuring viral safety. However, depending upon a choice of primer, there may be a case that not all the subtype of objective virus can be detected by this method, and, therefore, it is recommendable to evaluate, in advance, if subtype of a broad range can be detected.

NAT will be an effective evaluation method for virus removal capability at viral clearance studies. However, in case of viral inactivation process, viral inactivation obtained by this method may be underrated, since there is a case that inactivated virus still shows positive on nucleic acid. Further, at introduction of NAT, cautions should be taken on rationality of detection sensitivity, choice of a standard which is used as run-control, quality assurance and maintenance of a reagent used for primer, interpretation of positive and negative results, etc.

10. Reporting and preservation

All the items relating to virus test and viral clearance studies should be reported and preserved.

11. Others

The Notice, Iyakushin No. 329, should be used as a reference at virus test and viral clearance studies.

Conclusion

As mentioned at the Introduction, assurance of quality/safety etc. of JP listed drugs should be achieved by state-of-the-art methods and concepts reflecting the progress of science and accumulation of experiences.

The basis for ensuring viral safety of JP listed biotechnological/biological products is detailed in this General Information. What is discussed here is that an almost equal level of measures are required for both development of new drugs and for existing products as well, which means that similar level of concerns should be paid on both existing and new products in aspect of viral safety. This document is intended to introduce a basic concept that quality and safety assurance of JP listed product should be based upon the most advanced methods and concepts. This document has been written to cover all conceivable cases, which can be applied to all biotechnological/biological products. Therefore, there may be cases that it is not so rational to pursue virus tests and viral clearance studies in accordance with this document on each product, which has been used for a long time without any safety issue. So, it will be necessary to elaborate the most rational ways under a case-by-case principle taking into due consideration source, origin, type, manufacturing process, characteristics, usages at clinical stage, accumulation of the past usage record, etc. relating to such biotechnological/biological products.

Add the following:

18. Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices 39 of Japanese Pharmacopoeia and Other Standards

Introduction

The Official Gazette issued on March 29, 2002 announced that General Notices or General Rules of the Japanese Pharmacopoeia of the Standard of Drugs not required Approval, of the Biological Products Standard and of the Require-

ments for Antibiotic Products of Japan were amended to add a provision that ''When a drug product or a drug substance which is used to manufacture a drug product, is manufactured from a raw material of animal origin, the animal in question should be in principle a healthy subject, if not otherwise provided.''.

The Notice Iyaku-hatsu No. 0329001, which was issued on the same date, provided that ''Healthy subject herein provided is the animal which does not cause any disease or any infection to human being at an appropriate production process and use of the drug product, and as for the oral or external drug for example, the animal, as its raw material of animal origin, should be confirmed at this stage to meet the Food Standard. It has to be noted that this standard of healthy subject has to be revised timely taking into account the up-to-date information with respect to the amphixenosis infections common between human beings and animals.''.

This General Information describes safety assurance against infection of drugs, which are manufactured from raw materials of animal origin, to follow up the Notice as mentioned above.

1. Basic concept

When drugs derived from raw materials of animal origin including human are used, it is important to take into account any possibility that communicable disease agents such as virus may cause infectious disease or any possible hazards to patients. In such case, it goes without saying that the primary subject that has to be considered is the absence of any infectious agents such as virus in the raw materials of animal origin including human as the source of the drug. More important points are whether the drugs derived from such raw materials are free of such infectious agents and whether there is any possibility of transmission of infectious agents when the drugs are administered to patient. The eligibility of animals including human, as the source of raw materials of drugs, in other words ''the subject which is free from any disease or transmission of infectious agents that is infectious to human being at an appropriate production process and use of the drug product'' is that ''The drug should be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animals including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indications of the final product.''

2. Animals including human as the source of raw materials of drugs

What is the most clear and appropriate preventive measures against infection to human being due to administration of drugs which are derived from animals including human is to assure the absence of any infectious agents such as virus in its raw materials or an appropriate critical raw material by each of the followings: (1) the use of raw materials of healthy animal origin, which are proved to be free from communicable disease agents to human, or (2) the use of appropriate critical raw materials (e.g., cell substrate, blood plasma, pooled urine after some treatments) for drug production, which are proved to be free from communicable disease agents after certain appropriate processing on raw materials of animal origin.

As for raw materials of drugs of human origin, cell, tissue, blood, placenta, urine, etc. are used. Whenever it is sufficient and possible each donor, as the origin of such raw materials, should be asked his (her) health condition and undergoes his (her) medical examination at this stage, so that the appropriateness as a donor can be confirmed from the standpoint of safety concerning communicable disease agents such as virus.

For example, ''Basic concept on handling and use of a drug product, etc. which is derived from cell/tissue'' (Attachment 1 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000) and ''Guidance for quality and safety assurance of a drug product, etc. which is derived from human cell/tissue (Attachment 2 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000)'' issued by the Director-General of the Medicinal Safety Bureau, Ministry of Health and Welfare, states that since the cell/tissue supplied by a human donor comes to be applied to patients without processing through any sufficient inactivation or removal of communicable disease agents, the selection and qualification criteria on such donor has to be established. These criteria are to be composed with the respect to the check items on the case history and the physical conditions as well as the test items on the various transmission of infectious agents through cell/tissue, and that the appropriateness of these criteria has to be clarified. Hepatitis Type-B (HBV), Hepatitis Type-C (HCV), Human Immune Deficiency Viral infections (HIV), Adult T-Cell Leukemia and Parvovirus B19 Infections should be denied through the interview to the donor and the tests (serologic test, nucleic-acid amplification test, etc.). Further, if necessary, Cytomegalovirus infection and EB Virus infection should be denied by tests. ''Infections caused by bacteria such as Treponema pallidum, Chlamydia, Gonococci, Tubercule bacillus, etc.'', ''septicemia and its suspicious case'', ''vicious tumor'', ''serious metabolic or endocrine-related disorders'', ''collagenosis and haematological disorder'', ''hepatic disease'' and ''dementia (transmissible spongiform encephalopathies and its suspicious case)'' should be checked on the case history or by the interview, etc. and the experience of being transfused or/and transplanted should be checked to confirm eligibility as a donor. The most appropriate check items and test methods then available are to be used, which need to be reconsidered at appropriate timing taking into account the updated knowledge and the progress of the science and the technologies. At screening of a donor, reexaminations has to be made at appropriate timing using the eligible check items and the test methods taking into account the window period (Initial period after infection, in which antibody against bacteria, fungi or virus is not detected.)

In the case of plasma derivatives produced from the donated blood in Japan, the donor should be checked by means of self-assessed report about health conditions, and a serologic check and a nucleic acid amplification test (NAT) on 50 pooled plasma should be performed at the stage of do-

nated blood. Further, the plasma material (i.e., critical raw material) for fractionation should be stored 4 months in minimum so that the arrangement could be taken based on the information available after collection of the blood and the blood infusion to exclude the possibility of using any critical raw material which might cause infection to patients.

On the other hand, as for the materials such as urine which are taken from the unspecified number of the donors and come to be critical raw materials for drug production after some treatments, it is unrealistic and not practical to conduct the tests of virus infection, etc. on the individual donor. Consequently, appropriate tests such as virus test has to be performed on such critical raw materials for drug production.

In the case of the animals besides human, the wild ones should be excluded. Only the animals, which are raised under well sanitarily controlled conditions taken to prevent bacterial contamination or under the effective bacterial pollution monitoring systems, have to be used, and it is recommended that the animals from a colony appropriately controlled under specific pathogen-free (SPF) environment are to be used as far as possible. Further, for the animals regulated under the Food Standard, only the animals that met this standard should be used. It should be confirmed by appropriate tests that the animals were free from pathogen, if necessary.

The concrete measures to avoid transmittance or spread of infectivity of prion, which is considered to be the pathogen of transmissible spongiform encephalopathies (TSEs), as far as possible are the followings: ① avoidance of use of animals, which are raised in the areas where high incidence or high risk of TSEs (Scrapie in sheep and goat, bovine spongiform encephalopathies (BSE) in cattle, chronic wasting disease (CWD) in deer, new type of Creutzfeldt-Jacob-Disease (CJD) in human, etc.) is reported, and humans, who have stayed long time (more than 6 months) in such areas, as raw materials or related substances of drugs; ② avoidance of use of any substances that are derived from the individual infected with scrapie, BSE, CJD, etc.; ③ avoidance of using a material derived from organ, tissue and cell, etc. of high risk of TSEs; and ④ taking appropriate measures basing on the information collected, which includes incidence of TSEs, the results of epidemiological investigation and the experimental research on prion, and incidence of tardive infection on donors after collecting raw materials, etc.

3. Human or animal cells which are used as critical raw materials for drug production

Cell substrates derived from humans or animals are used for drug production. In such case, it is desirable that the humans or the animals, which are the origins of the cell substrates, are healthy subjects. However, it is considered practical that viral safety of the drugs derived from the cell substrates are evaluated on the cells, which are so called critical raw materials for production of such drugs. In such case, the safety should be confirmed through the test and analysis on established cell bank thoroughly with respect to virus etc., as far as possible. The items and the methods of the tests that have been followed in this case are described in detail in the Notice of Japanese version on the internationally accepted ICH Guideline entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare). In the meantime, it is important how to handle the cell in case that any virus has been detected under the cell level tests. This Notice describes how to cope with this situation as follows: ''It is recognised that some cell lines used for the manufacture of product will contain endogenous retroviruses, other viruses or viral sequences. In such circumstances, the action plan recommended for manufacturer is described in Section V (Rationale and action plan for viral clearance studies and virus tests on purified bulk) of the Notice. The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or for causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk.'' For example, it is well known that Type A-, R- and C-endogenous particles like retrovirus are observed in the cells of the rodents used most often for drug production. It is also known that they are not infectious to human and is not dangerous, and CHO cells are generally used for drug production. The established cell lines (e.g., NAMALWA Cell, BALL-1 Cell, etc.) derived from cancer patients are sometimes used, but through the thorough virus tests, etc., their safety are confirmed. The established cell lines are assumed to be safer than the primary cultured cells which are hard to conduct the thorough virus test.

4. Establishment and control of appropriate production process and adherence to the clinical indication of final product for safety assurance

Safety assurance against potential infections at only the level of animals that are source of raw materials of drugs is limited. Further, ''health of animal'' can not be defined univocally, and the various factors have to be taken into account. The final goal of this subject is to protect human from any infectious disease caused by drugs. Achieving this goal, the establishment and control of appropriate production processes of each drug and the adherence to the clinical indications of the final product are important.

As mentioned above, the rodent cells used most often for the production of the drugs are known to have endogenous retrovirus sometimes. The reason why such cells can be used for the production of the drugs is that multiple measures are applied for safety in the purification stages which include appropriate inactivation or removal processes. There are cases in which the production procedure involves intentional use of a virus or a microorganism. In this case, relevant measures capable of removing or inactivating of such virus or

microorganism are appropriately incorporated in the purification process, so that the risk of infection to human can be fully denied and its safety can be assured when it is used as a drug. Further, even in the case that it is difficult to clarify the risk of contamination of the infectious agents or that the raw material are contaminated by viruses etc., the raw material in question may be used for the production of drugs so long as appropriate inactivation or removal processes are introduced, their effectiveness can be confirmed and the safety can be assured by appropriate control of the manufacturing processes under GMP, etc.

5. Conclusion

The qualification of animals including human, as the source of raw materials of drugs, in other words ''the subject which does not cause any infectious diseases to human being at an appropriate production process and use of the drug product'' is that ''the drug has to be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animal including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indication of the final product.''

To cope with this subject, the advanced scientific measures, which actually reflect the updated knowledge and progress of the science and the technology about infectious diseases in human and infection of animal origin, have to be taken into account timely.

Add the following:

19. SDS-Polyacrylamide Gel Electrophoresis

The SDS-Polyacrylamide Gel Electrophoresis is used for the characterization of proteins in biotechnological and biological products and for control of purity and quantitative determinations.

This technique is a suitable analytical method with which to identify and to assess the homogeneity of proteins in biotechnological and biological products. The method is also routinely used for the estimation of protein subunit molecular masses and for determining the subunit compositions of purified proteins.

Ready-to-use gels and reagents are widely available on the market and can be used instead of those described in this text, provided that they give equivalent results and that they meet the validity requirements given below under Validation of the Test.

1. Characteristics of Polyacrylamide Gels

The sieving properties of polyacrylamide gels are afforded by the three-dimensional network of fibers and pores which is formed as the bifunctional bisacrylamide cross-links adjacent polyacrylamide chains. Polymerization is catalyzed by a free radical-generating system composed of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED).

As the acrylamide concentration of a gel increases, its effective pore size decreases. The effective pore size of a gel is operationally defined by its sieving properties; that is, by the resistance it imparts to the migration of macromolecules. There are limits on the acrylamide concentration that can be used. At high acrylamide concentrations, gels break much more easily and are difficult to handle. As the pore size of a gel decreases, the migration rate of a protein through the gel decreases. By adjusting the pore size of a gel, through manipulating the acrylamide concentration, the resolution of the method can be optimized for a given protein product. Thus, the physical characteristics of a given gel are determined by the relative concentrations of acrylamide and bisacrylamide, used in its preparation.

In addition to the composition of the gel, the state of the protein is an important determinant of the electrophoretic mobility. In the case of proteins, the electrophoretic mobility is dependent on the pK values of the charged groups and the size of the molecule. It is also influenced by the type, concentration and pH of the buffer, the temperature and the field strength, as well as by the nature of the support material.

2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions

The method cited as an example is limited to the analysis of monomeric polypeptides with a mass range of 14,000 to 100,000 daltons. It is possible to extend this mass range by various techniques (e.g., by using gradient gels, particular buffer systems, etc.), but those techniques are not discussed in this chapter.

Analysis by electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-Polyacrylamide Gel Electrophoresis) under denaturing conditions is the most common mode of electrophoresis used in assessing the quality of proteins in biotechnological and biological products, and will be the focus of the example described here. Typically, analytical electrophoresis of proteins is carried out in polyacrylamide gels under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind to SDS, become negatively charged and exhibit a consistent charge-to-mass ratio regardless of protein type. Because the amount of SDS bound is almost always proportional to the molecular mass of the polypeptide and is independent of its amino acid sequence, SDS-polypeptide complexes migrate through polyacrylamide gels with mobilities that are dependent on the size of the polypeptides.

The electrophoretic mobilities of the resultant SDS-polypeptide complexes all assume the same functional relationship to their molecular masses. Migration of SDS-complexes occurs toward the anode in a predictable manner, with low-molecular-mass complexes migrating faster than

larger ones. The molecular mass of a protein can therefore be estimated from its relative mobility calibrated in SDS-Polyacrylamide Gel Electrophoresis and the occurrence of a single band in such a gel is a criterion of purity.

However, modifications to the polypeptide backbone, such as N- or O-1inked glycosylation, have a significant impact on the apparent molecular mass of a protein, since SDS does not bind to a carbohydrate moiety in a manner similar to a polypeptide. Thus, a consistent charge-to-mass ratio is not maintained. The apparent molecular masses of proteins that have undergone post-translational modifications do not truly reflect the masses of the polypeptides.

1) Reducing conditions

Polypeptide subunits and three-dimensional structure of proteins are often fixed, at least in part, by the presence of disulfide bonds. A goal of SDS-Polyacrylamide Gel Electrophoresis under reducing conditions is to disrupt this structure by reducing the disulfide bonds. Complete denaturation and dissociation of proteins by treatment with 2-mercaptoethanol or dithiothreitol (DTT) will result in unfolding of the polypeptide backbone and subsequent complexation with SDS. Under these conditions, the molecular masses of the polypeptide subunits can be calculated by interpolation in the presence of suitable molecular-mass standards.

2) Non-reducing conditions

For some analyses, complete dissociation of the protein of interest into subunit peptides is not desirable. In the absence of treatment with reducing agents such as 2-mercaptoethanol or DTT, disulfide covalent bonds remain intact, preserving the oligomeric form of the protein. Oligomeric SDS-protein complexes migrate more slowly than their SDS-polypeptide subunits. In addition, non-reduced proteins may not be completely saturated with SDS and, hence, may not bind the detergent in the expected mass ratio. This makes molecular-mass determinations of these molecules by SDS-Polyacrylamide Gel Electrophoresis less straightforward than analyses of fully denatured polypeptides, since it is necessary that both standards and unknown proteins be in similar configurations for valid comparisons. However, the staining of a single band in such a gel is a criterion of purity.

3. Characteristics of Discontinuous Buffer System Gel Electrophoresis

The most widely used electrophoretic method for the analysis of complex mixtures of proteins involves the use of a discontinuous buffer system consisting of two contiguous, but distinct gels: a resolving or separating (lower) gel and a stacking (upper) gel. The two gels are cast with different porosities, pH, and ionic strengths. In addition, different mobile ions are used in the gel and electrode buffers. The buffer discontinuity acts to concentrate large-volume samples in the stacking gel, resulting in improved resolution. When power is applied, a voltage drop develops across the sample solution which drives the proteins into the stacking gel. Glycinate ions from the electrode buffer follow the proteins into the stacking gel. A moving boundary region is rapidly formed with the highly mobile chloride ions in the front and the relatively slow glycinate ions in the rear. A localized high-voltage gradient forms between the leading and trailing ion fronts, causing the SDS-protein complex to form into a very thin zone (called the stack) and to migrate between the chloride and glycinate phases. Regardless of the height of the applied sample solution in the wells, all SDS-protein complexes condense within broad limits and enter the resolving gel as a well-defined, thin zone of high protein density. The large-pore stacking gel does not retard the migration of most proteins and serves mainly as an anticonvective medium. At the interface of the stacking and resolving gels, the proteins experience a sharp increase in retardation due to the smaller pore size of the resolving gel. Once the proteins are in the resolving gel, their mobility continues to be slowed down by the molecular sieving effect of the matrix. The glycinate ions overtake the proteins, which then move in a space of uniform pH formed by the tris(hydroxymethyl)aminomethane and glycine. Molecular sieving causes the SDS-polypeptide complexes to separate on the basis of their molecular masses.

4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels

1) Assembling of the gel moulding cassette

Clean the two glass plates (size: e.g. 10 cm × 8 cm), the sample comb made of polytetrafluoroethylene, the two spacers and the silicone rubber tubing (diameter, e.g. 0.6 mm × 35 cm) with mild detergent and rinse extensively with water. Dry all the items with a paper towel or tissue. Lubricate the spacers and the silicone rubber tubing with non-silicone grease. Apply the spacers along each of the two short sides of the glass plate 2 mm away from the edges and 2 mm away from the long side corresponding to the bottom of the gel. Begin to lay the silicone rubber tubing on the glass plate by using one spacer as a guide. Carefully twist the silicone rubber tubing at the bottom of the spacer and follow the long side of the glass plate. While holding the silicone rubber tubing with one finger along the long side again twist the tubing and lay it on the second short side of the glass plate, using the spacer as a guide. Place the second glass plate in perfect alignment and hold the mould together by hand pressure. Apply two clamps on each of the two short sides of the mould. Carefully apply four clamps on the longer side of the gel mould, thus forming the bottom of the gel mould. Verify that the silicone rubber tubing is running along the edge of the glass plates and has not been extruded while placing the clamps.

2) Preparation of the gel

In a discontinuous buffer SDS polyacrylamide gel, it is recommended to pour the resolving gel, let the gel set, and then pour the stacking gel, since the compositions of the two gels in acrylamide-bisacrylamide, buffer and pH are different.

Preparation of the resolving gel: In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide for the resolving gel, using the values given in Table 1. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the tetramethylethylenediamine

(TEMED), filter the solution if necessary under vacuum through a cellulose acetate membrane (pore size: 0.45 μm); keep the solution under vacuum by swirling the filtration unit until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 1, swirl and pour immediately into the gap between the two glass plates of the mould. Leave sufficient space for the stacking gel (the length of the teeth of the sample comb plus 1 cm). Using a pipette, carefully overlay the solution with water-saturated isobutanol. Leave the gel in a vertical position at room temperature to allow polymerization to occur.

Preparation of the stacking gel: After polymerization is complete (about 30 minutes), pour off the isobutanol and wash the top of the gel several times with water to remove the isobutanol overlay and any unpolymerized acrylamide. Drain as much fluid as possible from the top of the gel, and then remove any remaining water with the edge of a paper towel.

In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide, using the values given in Table 2. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the TEMED, filter the solution if necessary under vacuum through a cellulose acetate membrane (pore size: 0.45 μm); keep the solution under vacuum by swirling the filtration unit until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 2, swirl and pour immediately into the gap between the two glass plates of the mould directly onto the surface of the polymerized resolving gel. Immediately insert a clean sample comb into the stacking gel solution, taking care to avoid trapping air bubbles. Add more stacking gel solution to fill completely the spaces of the sample comb. Leave the gel in a vertical position and allow to polymerize at room temperature.

3) Mounting the gel in the electrophoresis apparatus and electrophoretic separation

After polymerization is complete (about 30 minutes), remove the sample comb carefully. Rinse the wells immediately with water or with the running buffer for SDS-Polyacrylamide Gel Electrophoresis to remove any unpolymerized acrylamide. If necessary, straighten the teeth of the sample comb of the stacking gel with a blunt hypodermic needle attached to a syringe. Remove the clamps on one short side, carefully pull out the silicone rubber tubing and replace the clamps. Proceed similarly on the other short side. Remove the silicone rubber tubing from the bottom part of the gel. Mount the gel in the electrophoresis apparatus. Add the electrophoresis buffers to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates. This is best done with a bent hypodermic needle attached to a syringe. Never pre-run the gel before loading solutions, such as samples, since this will destroy the discontinuity of the buffer systems. Before loading solutions, such as samples, carefully rinse the stacking gel wells with the running buffer for SDS-Polyacrylamide Gel Electrophoresis. Prepare the test and reference solutions in the recommended sample buffer and treat as specified in the individual monograph. Apply the appropriate volume of each solution to the stacking gel wells. Start the electrophoresis using suitable operating conditions for the electrophoresis equipment to be used. There are commercially available gels of different surface area and thickness that are appropriate for various types of electrophoresis equipment. Electrophoresis running time and current/voltage may need to be altered depending on the type of apparatus used, in order to achieve optimum separation. Check that the dye front is moving into the resolving gel. When the dye is reaching the bottom of the gel, stop the electrophoresis. Remove the gel assembly from the apparatus and separate the glass plates. Remove the spacers, cut off and discard the stacking gel and immediately proceed with staining.

5. Detection of Proteins in Gels

Coomassie staining is the most common protein staining method, with a detection level of the order of 1 μg to 10 μg of protein per band. Silver staining is the most sensitive method for staining proteins in gels and a band containing l0 ng to 100 ng can be detected. All of the steps in gel staining are done at room temperature with gentle shaking in any convenient container. Gloves must be worn when staining gels, since fingerprints will stain.

1) Coomassie staining

Immerse the gel in a large excess of Coomassie staining TS and allow to stand for at least 1 hour. Remove the staining solution.

Destain the gel with a large excess of destaining TS. Change the destaining solution several times, until the stained protein bands are clearly distinguishable on a clear background. The more thoroughly the gel is destained, the smaller is the amount of protein that can be detected by the method. Destaining can be speeded up by including 2 to 3 g of anion-exchange resin or a small sponge in the destaining TS.

NOTE: the acid-alcohol solutions used in this procedure do not completely fix proteins in the gel. This can lead to losses of some low-molecular-mass proteins during the staining and destaining of the gel. Permanent fixation is obtainable by allowing the gel to stand in trichloroacetic acid TS for fixing for 1 hour before it is immersed in Coomassie staining TS.

2) Silver staining

Immerse the gel in a large excess of fixing TS and allow to stand for 1 hour. Remove the fixing solution, add fresh fixing solution and incubate either for at least 1 hour or overnight, if convenient. Discard the fixing solution and wash the gel in water for 1 hour. Soak the gel for 15 minutes in a 1 vol % glutaraldehyde solution. Wash the gel twice for 15 minutes in water. Soak the gel in fresh silver nitrate TS for silver staining for 15 minutes, in darkness. Wash the gel three times for 5 minutes in water. Immerse the gel for about 1 minute in developer TS until satisfactory staining has been obtained. Stop the development by incubation in blocking TS for 15 minutes. Rinse the gel with water.

6. Drying of Stained SDS-Polyacrylamide Gels

Depending on the staining method used, gels are pretreated in a slightly different way. For Coomassie staining, after the destaining step, allow the gel to stand in a diluted solution of concentrated glycerin (1 in 10) for at least 2 hours (overnight incubation is possible). For silver staining, add to the final rinsing a step of 5 minutes in a diluted solution of concentrated glycerin (1 in 50).

Immerse two sheets of porous cellulose film in water and incubate for 5 to 10 minutes. Place one of the sheets on a drying frame. Carefully lift the gel and place it on the cellulose film. Remove any trapped air bubbles and pour 2 to 3 mL of water around the edges of the gel. Place the second sheet on top and remove any trapped air bubbles. Complete the assembly of the drying frame. Place in an oven or leave at room temperature until dry.

7. Molecular-Mass Determination

Molecular masses of proteins are determined by comparison of their mobilities with those of several marker proteins of known molecular mass. Mixtures of proteins with precisely known molecular masses blended for uniform staining are commercially available for calibrating gels. They are obtainable in various molecular mass ranges. Concentrated stock solutions of proteins of known molecular mass are diluted in the appropriate sample buffer and loaded on the same gel as the protein sample to be studied.

Immediately after the gel has been run, the position of the bromophenol blue tracking dye is marked to identify the leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front. After staining, measure the migration distances of each protein band (markers and unknowns) from the top of the resolving gel. Divide the migration distance of each protein by the distance traveled by the tracking dye. The normalized migration distances so obtained are called the relative mobilities of the proteins (relative to the dye front) and conventionally denoted as *R*f. Construct a plot of the logarithm of the relative molecular masses (M_r) of the protein standards as a function of the *R*f values. Note that the graphs are slightly sigmoid. Unknown molecular masses can be estimated by linear regression analysis or interpolation from the curves of log M_r against *R*f as long as the values obtained for the unknown samples are positioned along the linear part of the graph.

8. Suitability of the Test (Validation)

The test is not valid unless the front end of the molecular mass marker migrates 80% of the migrating distance of the dye, and over the required separation range (e.g., the range covering the product and its dimer or the product and its related impurities) the separation obtained for the relevant protein bands shows a linear relationship between the logarithm of the molecular mass and the *R*f as described in 7. Additional requirements with respect to the solution under test may be specified in individual monographs.

9. Quantification of Impurities

Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity should be prepared by diluting the test solution. For example, where the limit is 5%, a reference solution would be a 1:20 dilution of the test solution. No impurity (any band other than the main band) in the electrophoretogram obtained with the test solution may be more intense than the main band obtained with the reference solution.

Under validated conditions, impurities may be quantified by normalization to the main band, using an integrating densitometer. In this case, the responses must be validated for linearity.

Test solutions:

Coomassie staining TS Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Developer TS Dissolve 2 g of citric acid monohydrate in water to make 100 mL. To 2.5 mL of this solution add 0.27 mL of formaldehyde solution and water to make 500 mL.

Fixing TS To 250 mL of methanol add 0.27 mL of formaldehyde solution and water to make 500 mL.

Silver nitrate TS for silver staining To 40 mL of sodium hydroxide TS add 3 mL of ammonia solution (28), then add dropwise 8 mL of a solution of silver nitrate (1 in 5) while stirring, and add water to make 200 mL.

Destaining TS A mixture of water, methanol and acetic acid (100) (5:4:1).

Blocking TS To 10 mL of acetic acid (100) add water to make 100 mL.

Trichloroacetic acid TS for fixing Dissolve 10 g of trichloroacetic acid in a mixture of water and methanol (5:4) to make 100 mL.

Table 1. Preparation of resolving gel

Solution components	Component volumes (mL) per gel mould volume of							
	5 mL	**10 mL**	**15 mL**	**20 mL**	**25 mL**	**30 mL**	**40 mL**	**50 mL**
6% Acrylamide								
Water	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
Acrylamide solution(1)	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 mol/L Tris solution (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Acrylamide								
Water	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
Acrylamide solution(1)	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 mol/L Tris solution (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Acrylamide								
Water	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
Acrylamide solution(1)	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 mol/L Tris solution (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Acrylamide								
Water	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
Acrylamide solution(1)	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 mol/L Tris solution (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
14% Acrylamide								
Water	1.4	2.7	3.9	5.3	6.6	8.0	10.6	13.8
Acrylamide solution(1)	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
1.5 mol/L Tris solution (pH 8.8)(2)	1.2	2.5	3.6	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Acrylamide								
Water	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
Acrylamide solution(1)	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 mol/L Tris solution (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution

(2) 1.5 mol/L Tris solution (pH 8.8): 1.5 mol/L tris-hydrochloride buffer solution, pH 8.8

(3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate

(4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.

(5) TEMED: *N*,*N*,*N*′,*N*′-tetramethylethylenediamine

Table 2. Preparation of stacking gel

Solution components	Component volumes (mL) per gel mould volume of							
	1 mL	**2 mL**	**3 mL**	**4 mL**	**5 mL**	**6 mL**	**8 mL**	**10 mL**
Water	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
Acrylamide solution(1)	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 mol/L Tris solution (pH 6.8)(2)	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
100 g/L SDS(3)	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
100 g/L APS(4)	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED(5)	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution

(2) 1.0 mol/L Tris solution (pH 6.8): 1 mol/L tris-hydrochloride buffer solution, pH 6.8

(3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate

(4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.

(5) TEMED: *N*,*N*,*N*′,*N*′-tetramethylethylenediamine

The Ministry of Health, Labour and Welfare
The Ministerial Notification on Partial Revision of the Japanese Pharmacopoeia (No. 151, March 2002)

In accordance with the provision of Paragraph 1 of Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 111, March 2001) as follows and enforce the revision on April 1, 2002. Although the revision shall come into effect on April 1, 2002, in the case of the drugs, which are prepared or imported before March 31, 2003, the previous texts in the General Notices of the Part I or the Part II in the Japanese Pharmacopoeia may be applied. (The paragraphs on the General Notices of the Part I are referred to the General Notices of the Part II.)

March 29, 2002

Chikara Sakaguchi
The Minister of Health, Labour and Welfare

Add the following paragraph to the General Notices of the Part I :

39. In principle, unless otherwise specified, animals used as a source of materials for preparing pharmaceutical preparations listed in the Japanese Pharmacopoeia must be healthy.

Delete the following Monograph:

Part I

Phenacetin

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