SUPPLEMENT II
TO
THE JAPANESE PHARMACOPOEIA
FIFTEENTH EDITION

Official From October 1, 2009

English Version

THE MINISTRY OF HEALTH, LABOUR AND WELFARE

Notice: This *English Version* of the Japanese Pharmacopoeia is published for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

The Ministry of Health, Labour and Welfare Ministerial Notification No. 425

Pursuant to Paragraph 1, Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 285, 2006) as follows*, and the revised Japanese Pharmacopoeia shall come into effect on October 1, 2009. However, in the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as "previous Pharmacopoeia") [limited to those listed in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as "new Pharmacopoeia")] and drugs which have been approved as of October 1, 2009 as prescribed under Paragraph 1, Article 14 of the same law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the Pharmaceutical Affairs Law (hereinafter referred to as "drugs exempted from approval")], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on March 31, 2011. In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and drugs which have been approved as of October 1, 2009 as prescribed under Paragraph 1, Article 14 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on March 31, 2011.

Akira Nagatsuma
The Minister of Health, Labour and Welfare

September 30, 2009

(The text 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 Food 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 of Supplement II to the Japanese Pharmacopoeia Fifteenth Edition from General Notice to Ultraviolet-visible Reference Spectra (pp. 2041 - 2276).

CONTENTS

PREFACE

The 15th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No. 285 of the Ministry of Health, Labour and Welfare (MHLW) on March 31, 2006.

In July 2006, the Committee on JP established the basic principles for the preparation of the JP 16th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

At the above Committee, the five basic principles of JP, which we refer to as the "five pillars" were established as follows: 1) Including all drugs which are important from the viewpoint of health care and medical treatment; 2) Making qualitative improvement by introducing the latest science and technology; 3) Promoting internationalization; 4) Making prompt partial revision as necessary and facilitating smooth administrative operation; and 5) Ensuring transparency regarding the revision, and disseminating the JP to the public. It was agreed that the Committee 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 measurements, including getting the understanding and cooperation of other parties concerned.

It was agreed that the JP should provide an official standard, being required to assure the quality of medicines in Japan in response to the progress of science and technology and medical demands at the time. It should define the standards for specifications, as well as the methods of testing to assure overall quality of all drugs in principle, and it should have a role in clarifying the criteria for quality assurance of drugs that are recognized to be essential for public health and medical treatment.

The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, the JP should have the characteristics of an official standard, which might be widely used by all parties concerned. It should provide information and understanding about the quality of drugs to the public, and it should be conducive to smooth and effective regulatory control of the quality of drugs, as well as promoting and maintaining international consistency and harmonization of technical requirements.

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.

The target date for the publication of JP 16th Edition (the Japanese edition) was set as April 2011.

JP Expert Committees are organized with the following panels: Panel on the Principles of Revisions; Sub-committee on the Principles of Revisions; Panel on Medicinal Chemicals; Panel on Antibiotics; Panel on Biologicals; Panel on Crude Drugs; Panel on Pharmaceutical Excipients; Panel on Physico-Chemical Methods; Panel on Preparations; Panel on Physical Methods; Panel on Biological Tests; Panel on Nomenclature; Panel on International Harmonization; Panel on Pharmaceutical Water; and Panel on Reference Standards. Furthermore, working groups are established under the Panel on Physico-Chemical Methods, Panel on Preparations and Panel on Biological Tests to expedite discussion of revision drafts of Monographs.

In the Committee on JP, Takao Hayakawa took the role of chairman from July 2003 to September 2009.

In addition to the regular revision every five years in line with the basic principles for the preparation of the JP it was agreed that partial revision should be done as necessary to take account of recent progress of science and in the interests of international harmonization.

In accordance with the above principles, the panels initiated deliberations on selection of articles, and on revisions for General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests, Monographs and so on.

Draft revisions covering subjects in General Rules for Crude Drugs, General Tests and Monographs, for which discussions were finished between April 2007 and March 2009, were prepared for a supplement to the JP 15. They were examined by the Committee on JP in April 2009, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2009, and then submitted to the Minister of MHLW.

Numbers of discussions in the panels to prepare the supplement drafts were as follows: Panel on Principles of Revisions (3); Panel on Medicinal Chemicals (23); Panel on Antibiotics (8); Panel on Biologicals (8); Panel on Crude Drugs (21); Panel on Pharmaceutical Excipients (10): Panel on Physico-Chemical Methods (11, including the working groups); Panel on Preparations (19, including the working groups); Panel on Physico-Chemical Methods (9): Panel on Biological Tests (9); Panel on Nomenclature (6); Panel on Inter-

national Harmonization (3); and Panel on Pharmaceutical Water (8).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer's Association of Osaka and of Tokyo, the Tokyo Crude Drugs Association, the Japan Pharmaceutical Excipients Council, the Home Medicine Association of Japan, the Japan Kampo Medicine Manufacturers' Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Parental Drug Association, the Japan Reagent Association, the Japan Oilseeds Processors Association, and the Association of Membrane Separation Technology of Japan.

In consequence of this revision, the JP 15th Edition carries 1673 articles, owing to the addition of 106 articles and the deletion of 1 article.

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

1. The Supplement II to JP 15th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Rules for Crude Drugs; General Tests, Official Monographs; Infrared Reference Spectra; and Ultraviolet-visible Reference Spectra; then followed by General Information; and as an appendix a Cumulative Index containing references to the main volume and the Supplements I and II.

2. The articles in General Rules for Preparations, Official Monographs, 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/Description of crude drugs
(13) Identification tests
(14) Specific physical and/or chemical values
(15) Purity tests
(16) Loss on drying or ignition, or water
(17) Residue on ignition, total ash or acid-insoluble ash
(18) Tests being required for pharmaceutical preparations and other 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, 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) Infrared and/or ultraviolet-visible absorption spectrometry
(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) Acidity

(6) Alkalinity
(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) Residual solvent
(38) Other impurities
(39) Readily carbonizable substances

7. The following articles were newly added to the General Rules for Crude Drugs:
(1) Pogostemon Herb
(2) Nutmeg
(3) Quercus Bark
(4) Longan Aril
(5) Royal Jelly

8. The following article was deleted from the General Rules for Crude Drugs:
(1) Rice Starch

9. The following item was newly added to the General Tests, Processes and Apparatus:
(1) 2.04 Amino Acid Analysis of Proteins

10. The following items of the General Tests, Processes and Apparatus were revised:
(1) 1.07 Heavy Metals Limit Test
(2) 1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)
(3) 1.09 Qualitative Tests
(4) 2.01 Liquid Chromatography
(5) 3.01 Determination of Bulk and Tapped Densities
(6) 3.02 Specific Surface Area by Gas Adsorption
(7) 3.03 Powder Particle Density Determination
(8) 3.04 Particle Size Determination
(9) 7.02 Test Methods for Plastic Containers

11. The following Reference Standards were newly added:
Aciclovir
Calcitonin (Salmon)
Danazol
Diflucortolone Valerate
Doxazosin Mesilate
Fludrocortisone Acetate
Flutamide
Gefarnate
D-Glucuronolactone
Indapamide
Ipriflavone
Losartan Potassium
Pioglitazone Hydrochloride
Prazosin Hydrochloride
Probucol
Sevoflurane
Simvastatin
Tacrolimus Hydrate
Tazobactam
Teprenone
Tosulfloxacin Tosilate Hydrate
Troxipide

12. The following Reference Standards were revised:
Amlodipine Besilate
Amoxicillin
Azathioprine
Cefalexin
Cefatrizine Propylene Glycolate
Cefixime
Cefroxadine Hydrate
Cefteram Pivoxil Mesitylenesulfonate
Clomifene Citrate
Diethylcarbamazine Citrate
Ethenzamide
Faropenem Sodium
Griseofulvin
Minocycline Hydrochloride
Pivmecillinam Hydrochloride

Probenecid
Prochlorperazine Maleate
Warfarin Potassium

13. English and Latin titles of drugs were based, in principle, on the International Nonproprietary Names for Pharmaceutical Substances, and the chemical names were based on the Rules of the International Union of Pure and Applied Chemistry (IUPAC).

14. 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.

15. Structural formula of drug represents, as far as possible, the steric configuration.

16. The test procedures in monographs were written in full, except within the same monograph and in the monographs for preparation having a corresponding monograph of their principal material substances.

17. The following monographs were added:
Acemetacin Capsules
Acemetacin Tablets
Aciclovir
L-Alanine
Allopurinol Tablets
Amiodarone Hydrochloride
Amiodarone Hydrochloride Tablets
Amlodipine Besilate Tablets
Amoxicillin Capsules
Aprindine Hydrochloride
Aprindine Hydrochloride Capsules
Argatroban Hydrate
Azelastine Hydrochloride Granules
Betaxolol Hydrochloride
Cadralazine
Cadralazine Tablets
Calcitonin (Salmon)
Cefalexin Capsules
Cefalexin for Syrup
Cefatrizine Propylene Glycolate for Syrup
Cefixime Capsules
Cefroxadine for Syrup
Cefteram Pivoxil Tablets
Cinoxacin
Cinoxacin Capsules
Clebopride Malate
Danazol
Dibekacin Sulfate Ophthalmic Solution
Diflucortolone Valerate
Doxazosin Mesilate
Droxidopa
Droxidopa Capsules
Doroxidopa Fine Granules
Ecabet Sodium Granules
Ecabet Sodium Hydrate
Emorfazone Tablets
Fludrocortisone Acetate
Flutamide
Flutoprazepam
Flutoprazepam Tablets
Furosemide Injection
Gefarnate
Gentamicin Sulfate Ophthalmic Solution
Gliclazide
Heparin Calcium
Imidapril Hydrochloride
Imidapril Hydrochloride Tablets
Indapamide
Indapamide Tablets
Ipriflavone
Ipriflavone Tablets
Irsogladine Maleate
Irsogladine Maleate Fine Granules
Irsogladine Maleate Tablets
Isepamicin Sulfate Injection
Ketoconazole
Ketoconazole Cream
Ketoconazole Lotion
Ketoconazole Solution
Levofloxacin Hydrate
Lincomycin Hydrochloride Injection
Losartan Potassium
L-Lysine Acetate
Meropenem for Injection
Minocycline Hydrochloride Tablets
Mosapride Citrate Hydrate
Mosapride Citrate Tablets
Pimozide
Pioglitazone Hydrochloride
Pivmecillinam Hydrochloride Tablets
Prazosin Hydrochloride
Prednisolone Sodium Phosphate
Probucol
Propafenone Hydrochloride
Propafenone Hydrochloride Tablets
Rebamipide
Rebamipide Tablets
Sevoflurane
Simvastatin
Purified Sodium Hyaluronate
Sodium Valproate Syrup
Sodium Valproate Tablets

Streptomycin Sulfate for Injection
Sulindac
Tacrolimus Hydrate
Tazobactam
Teprenone
Tiapride Hydrochloride
Tiapride Hydrochloride Tablets
Tosufloxacin Tosilate Hydrate
Tosufloxacin Tosilate Tablets
Troxipide
Troxipide Fine Granules
Troxipide Tablets
Ubenimex Capsules
Ursodeoxycholic Acid Granules
Ursodeoxycholic Acid Tablets
Zolpidem Tartrate
Goshajinkigan Extract
Hachimijiogan Extract
Longan Aril
Nutmeg
Pogostemon Herb
Quercus Bark
Royal Jelly
Shimbuto Extract

18. The following monographs were revised:
Dried Aluminum Hydroxide Gel Fine Granules
Aminophylline Injection
Amoxicillin Hydrate
Azathioprine Tablets
Betamethasone Tablets
Carmellose
Carmellose Calcium
Carmellose Sodium
Cefaclor Compound Granules
Cellacefate
Microcrystalline Cellulose
Powdered Cellulose
Clindamycin Hydrochloride
Clindamycin Hydrochloride Capsules
Clomifene Citrate Tablets
Codeine Phosphate Tablets
Corn Starch
Wood Creosote
Croscarmellose Sodium
Diethylcarbamazine Citrate Tablets
Digoxin
Digoxin Injection
Digoxin Tablets
Dimenhydrinate Tablets
Distigmine Bromide Tablets
Ephedrine Hydrochloride Tablets
Ergometrine Maleate Injection
Erythromycin Enteric-Coated Tablets
Estradiol Benzoate Injection (Aqueous Suspension)
Estriol Injection (Aqueous Suspension)
Ethenzamide
Ethinylestradiol
Famotidine Powder
Faropenem Sodium for Syrup
Faropenem Sodium Tablets
Flopropione
Fosfomycin Sodium for Injection
Griseofulvin Tablets
Heparin Sodium
Human Menopausal Gonadotrophin
Hydralazine Hydrochloride for Injection
Hydrocortisone Sodium Phosphate
Hydroxypropylcellulose
Low Substituted Hydroxypropylcellulose
Hypromellose
Hypromellose Phthalate
Indigocarmine Injection
Indometacin Suppositories
Isoniazid Injection
Isoniazid Tablets
Isosorbide Dinitrate Tables
Josamycin Tablets
Anhydrous Lactose
Lactose Hydrate
Lidocaine Injection
Meglumine Iotalamate Injection
Meglumine Sodium Amidotrizoate Injection
Mepivacaine Hydrochloride Injection
Methylcellulose
Minocycline Hydrochloride for Injection
Morphine Hydrochloride Injection
Morphine Hydrochloride Tablets
Nicomol Tablets
Norethisterone
Phenobarbital
10% Phenobarbital Powder
Phenolsulfonphthalein Injection
Phenytoin Powder
Phenytoin Tablets
Pipemidic Acid Hydrate
Potato Starch
Probenecid Tablets
Procainamide Hydrochloride
Procainamide Hydrochloride Injection
Procainamide Hydrochloride Tablets
Prochlorperazine Maleate Tablets
Progesterone
Progesterone Injection
Propylthiouracil Tablets
Protamine Sulfate Injection

Rice Starch
Rokitamycin Tablets
Sodium Iotalamate Injection
Sodium Valproate
Teicoplanin
Testosterone Enanthate Injection
Testosterone Propionate Injection
Tolbutamide Tablets
Ursodeoxycholic Acid
Vasopressin Injection
Warfarin Potassium Tablets
Wheat Starch
Zinc Sulfate Hydrate
Apricot Kernel
Astragalus Root
Bear Bile
Bupleurum Root
Calumba
Powdered Calumba
Cardamon
Cornus Fruit
Daiokanzoto Extract
Eleutherococcus Senticosus Rhizome
Hochuekkito Extract
Japanese Valerian
Powdered Japanese Valerian
Kakkonto Extract
Lithospermum Root
Longgu
Nuphar Rhizome
Peach Kernel
Powdered Peach Kernel
Perilla Herb
Polygonatum Rhizome
Saposhnikovia Root
Scopolia Rhizome
Senega
Powdered Senega
Turmeric
Powdered Turmeric
Zanthoxylum Fruit
Powdered Zanthoxylum Fruit

19. The following monograph was deleted:
Meglumine Amidotrizoate Injection

Those who were engaged in the preparation of Supplement II to JP 15 are as follows:

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Takeshi Yamazaki
Toshiyasu Yamada
Keiichi Yamamoto
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Tosuke Yamamoto
Sumie Yoshioka
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Etsuo Yonemochi
Chikako Yomota
Masaaki Wada
Eiji Watanabe
Haruo Watanabe

*: Chairman, the Committee on JP
**: Acting Chairman, the Committee on JP

Supplement II to The Japanese Pharmacopoeia Fifteenth Edition

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 Crude Drugs Test are applicable to the following:

Acacia, Achyranthes Root, Agar, Akebia Stem, Alisma Rhizome, Aloe, Alpinia Officinarum Rhizome, Amomum Seed, Anemarrhena Rhizome, Angelica Dahurica Root, Apricot Kernel, Aralia Rhizome, Areca, Artemisia Capillaris Flower, Asiasarum Root, Asparagus Tuber, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benincasa Seed, Benzoin, Bitter Cardamon, Bitter Orange Peel, Bupleurum Root, Burdock Fruit, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Chrysanthemum Flower, Cimicifuga Rhizome, Cinnamon Bark, Citrus Unshiu Peel, Clematis Root, Clove, Cnidium Monnieri Fruit, Cnidium Rhizome, Coix Seed, Condurango, Coptis Rhizome, Cornus Fruit, Corydalis Tuber, Crataegus Fruit, Cyperus Rhizome, Digenea, Dioscorea Rhizome, Dolichos Seed, Eleutherococcus Senticosus Rhizome, Ephedra Herb, Epimedium 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, Honey, Houttuynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Jujube, Jujube Seed, Leonurus Herb, Lilium Bulb, Lindera Root, Lithospermum Root, Longan Aril, Longgu, Lonicera Leaf and Stem, Loquat Leaf, Lycium Bark, Lycium Fruit, Magnolia Bark, Magnolia Flower, Mallotus Bark, Mentha Herb, Moutan Bark, Mulberry Bark, Nelumbo Seed, Notopterygium Rhizome, Nuphar Rhizome, Nutmeg, Nux Vomica, Ophiopogon Tuber, Oriental Bezoar, Oyster Shell, Panax Japonicus Rhizome, Peach Kernel, Peony Root, Perilla Herb, Peucedanum Root, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Pogostemon Herb, Polygala Root, Polygonatum Rhizome, Polygonum Root, Polyporus Sclerotium, Poria Sclerotium, Powdered Acacia, Powdered Agar, 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 Corydalis Tuber, Powdered Cyperus Rhizome, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Longgu, Powdered Magnolia Bark, Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Japonicus Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polypourus Sclerotium, Powderd Poria Sclerotium, Powdered Processed Aconite Root, 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 Turmeric, Powdered Zanthoxylum Fruit, Processed Aconite Root, Processed Ginger, Prunella Spike, Pueraria Root, Quercus Bark, Red Ginseng, Rehmannia Root, Rhubarb, Rose Fruit, Rosin, Royal Jelly, Safflower, Saffron, Saposhnikovia Root, Sappan Wood, Saussurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sinomenium Stem, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Turmeric, Toad Venom, Tragacanth, Tribulus Fruit, Trichosanthes Root, Uncaria Hook, Zanthoxylum Fruit, Zedoary.

GENERAL TESTS, PROCESS AND APPARATUS

Change the introduction to read:

General Tests, Processes and Apparatus includes common methods for tests, useful test methods for quality recognition of drugs and other articles related to them. Unless otherwise specified, acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, amino acid analysis of proteins, ammonium determination, arsenic determination, atomic absorption spectrophotometry, boiling point determination, chloride determination, conductivity measurement, congealing point determination, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, distilling range determination, endpoint determination in titrimetry, flame coloration, fluorometry, foreign insoluble matter test for injetcions, foreign insoluble matter test for ophthalmic solutions, gas chromatography, heavy metal 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, microbial assay for antibiotics, melting point determination, methanol determination, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolarity determination, oxygen flask combustion method, particle size determination, particle size distribution test for preparations, pH determination, powder particle density determination, qualitative test, refractive index determination, residual solvents test, residue on ignition determination, specific gravity and density determination, specific surface area determination, sulfate determination, test for bacterial endotoxins, test for glass containers for injections, test for metal particles in ophthalmic ointments, test for microbial limit, test for microbial limit for crude drugs, test for plastic containers, test for pyrogen, test for readily carbonizable substances, test for rubber closure for aqueous infusions, test for sterility, test for total organic carbon, test of extractable volume for injection , thermal analysis, thin-layer chromatography, ultraviolet-visible spectrophotometry, uniformity of dosage units (test for content uniformity, mass variation test), viscosity determination, vitamin A assay, 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 Fats and Fatty Oils Test, and sampling, preparation of sample for analysis, microscopic examination, purity test, loss on drying, total ash, acid-insoluble ash, extract content, and essential oil content of crude drugs are performed as directed in the corresponding items under Crude Drugs Test.

The number of each test method is a category number given individually. The number in blackets (< >) appeared in monograph indicates the number corresponding to the general test method.

1.07 Heavy Metals Limit Test

Change (3) Method 3 under the Preparation of test solutions and control solutions to read:

(3) Method 3

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then incinerate by ignition between 500ºC and 600ºC. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)

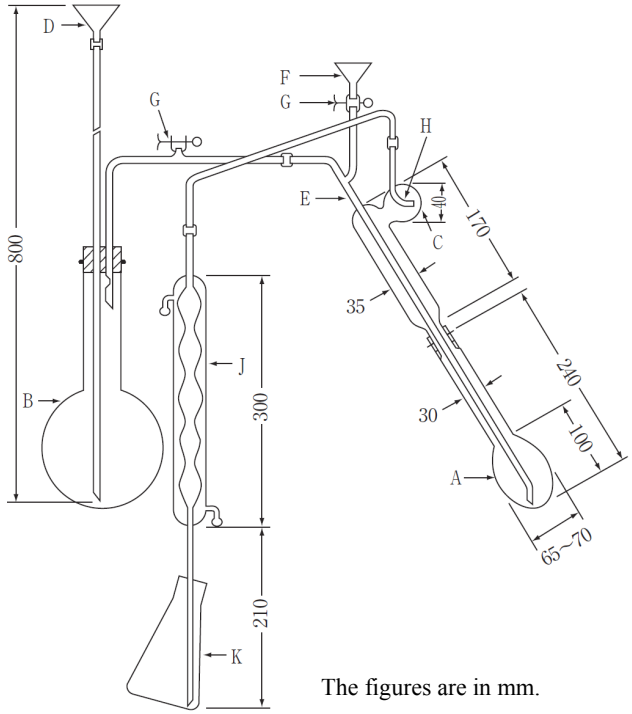
Change the following to read:

Nitrogen Determination is a method to determine nitrogen in an organic substance in which the nitrogen is converted into ammonia nitrogen by thermal decomposition of the organic substance with sulfuric acid, and the ammonia liberated by alkali and trapped by distillation with steam is determined by titration.

1. Apparatus

Use the apparatus illustrated in Fig. 1.08-1. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Alternatively, apparatus can be used in which some of the procedures, such as digestion of organic substances, distillation of the liberated ammonia, and endpoint detection methods in titrimetry (e.g., potentiometric titration or titration by colorimeter) are automated.



The figures are in mm.

A: Kjeldahl flask
B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling tips for preventing bumping have been added
C: Spray trap
D: Water supply funnel
E: Steam tube
F: Funnel for addition of alkali solution to flask A
G: Rubber tubing with a clamp
H: A small hole having a diameter approximately equal to that of the delivery tube
J: Condenser, the lower end of which is beveled
K: Absorption flask

Fig. 1.08-1

2. System suitability

If an automated apparatus is used, it is necessary to confirm periodically the suitability of the apparatus according to the following method:

Weigh accurately about 1.7 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours, dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, and transfer to an digestion flask. When the test is, perform as directed in the instrumental manual.: the nitrogen content (%) in amidosulfuric acid should be determined between 14.2% and 14.6%.

3. Reagents, Test Solutions

Decomposition Accelerators Unless otherwise specified, use 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of cupper (II) sulfate pentahydrate. The composition and amount of the digestion accelerator may be modified if it is confirmed that the modified one give almost the same results using the sample as those obtained from the conventional catalyst.

4. Procedure

Usually, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flaks A. Add the decomposition accelerator and wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus (Fig 1.08-1) washed beforehand by passing stream through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, lose the clamp attached to the rubber tubing G, then begin the distillation with stream, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J, rinsing the end part with a small quantity of water, and titrate <*2.50*> the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS = 0.1401 mg of N

If an automated apparatus is used, proceed as directed in the instrumental procedure.

1.09 Qualitative Tests

Change (2) under the Phosphate (Orthophosphate) to read:

Phosphate (Orthophosphate)

(2) Acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with hexaammonium heptamolybdate TS on warming. The precipitate dissolves upon subsequent addition of sodium hydroxide TS or ammonia TS.

2.01 Liquid Chromatography

Change the Apparatus to read:

Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few μg. The recorder is used to record the output signals of the detector. As required, a data processor may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a stepwise or gradient fashion.

Change the System Suitability to read:

System Suitability

System suitability testing is an integral part of test methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. System suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test method of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

In system suitability testing of the chromatographic systems, the evaluation of "System performance" and "System repeatability" is usually required. For quantitative purity tests, the evaluation of "Test for required detectability" may also be required.

(1) Test for required detectability

For purity tests, when it is confirmed that the target impurity is distinctly detected at the concentration of its specification limit, it is considered verified that the system used has adequate performance to achieve its intended use.

For quantitative purity tests, "Test for required detectability" is usually required, and in order to confirm, in some degree, the linearity of response near its specification limit, the range of expected response to the injection of a certain volume of target impurity solution at the concentration of its specification limit should be prescribed. For limit test, "Test for required detectability" is not required, if the test is performed by comparing the response from sample solution with that from standard solution at the concentration of its specification limit. "Test for required detectability" is also not required, if it is confirmed that the impurity can be detected at its specification limit by the evaluation of "System repeatability" or some other procedure.

(2) System performance

When it is confirmed that the specificity for determining the test ingredient is ensured, it is considered verified that the system used has adequate performance to achieve its intended use.

In assay, "System performance" should be defined by the resolution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable), and when appropriate, by their order of elution. In purity tests, both the resolution and the order of elution between the test ingredient and a target substance to be separated (a closely eluting compound is preferable) should be prescribed. In addition, if necessary, the symmetry factor of the test ingredient should be prescribed together with them. However, if there is no suitable target substance to be separated, it is acceptable to define "System performance" using the number of theoretical plates and the symmetry factor of the test ingredient.

(3) System repeatability

When it is confirmed that the degree of variation (precision) of the response of the test ingredient is at a level that meets the requirement of "System repeatability", it is considered verified that the system used has adequate performance to achieve its intended use.

The allowable limit of "System repeatability" is normally defined as the relative standard deviation (*RSD*) of the response of the test ingredient in replicate injections of standard solution. It is acceptable to confirm the repeatability of the system not only by replicate injections of standard solution before sample injections, but also by divided injections of standard solution before and after sample injections, or by interspersed injections of standard solution among sample injections.

In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of "System repeatability" which can guarantee a level of "System repeatability" equivalent to that at 6 replicate injections.

The allowable limit of "System repeatability" should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test.

2.04 Amino Acid Analysis of Proteins

Amino acid analysis of proteins refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

1. Hydrolysis of Protein and Peptide

Acid hydrolysis at 110°C for 24 hours using 6 mol/L hydrochloric acid containing phenol (Method 1) is the most common method for hydrolyzing protein and samples. The result should be analyzed carefully because several amino acids are chemically modified during the acid hydrolysis and thus not recovered quantitatively. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Peptide bonds involving isoleucine and valine are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively.

The hydrolysis techniques, Methods 2 to 11, are used to address these concerns. Some of the hydrolysis techniques, Methods 4 to 11, may cause modifications of cysteine, methionine, asparagines and glutamine to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis by Method 1.

(i) Method 1: Hydrolysis using hydrochloric acid containing phenol (liquid phase hydrolysis, vapor phase hydrolysis)

Prevention of tryptophan oxidation

(ii) Method 2: Mercaptoethanesulfonic acid hydrolysis (vapor phase hydrolysis)

(iii) Method 3: Hydrolysis using hydrochloric acid containing thioglycolic acid (vapor phase hydrolysis)

Cysteine-cystine and methionine oxidation

(iv) Method 4: Hydrolysis by Method 1 or Method 2 after oxidation with performic acid

Cysteine-cystine oxidation

(v) Method 5: Hydrolysis using hydrochloric acid containing sodium azide (liquid phase hydrolysis)

(vi) Method 6: Hydrolysis using hydrochloric acid containing dimethylsulfoxide (vapor phase hydrolysis)

Cysteine-cystine reduction and alkylation

(vii) Method 7: Hydrochloric acid hydrolysis after a vapor phase pyridylethylation reaction

(viii) Method 8: Hydrochloric acid hydrolysis after a liquid phase pyridylethylation reaction

(ix) Method 9: Hydrochloric acid hydrolysis after a liquid phase carboxymethylation reaction

Conversion of cysteine-cystine to mixed disulfide

(x) Method 10: Hydrochloric acid hydrolysis after a reaction with dithiodiglycolic acid or dithiodipropionic acid

Conversion of asparagine and glutamine

(xi) Method 11: Hydrochloric acid hydrolysis after reaction with bis(1,1-trifuoroacetoxy) iodobenzene

A time-course study is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. This technique will allow the analyst to account for some residue destruction.

Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

2. Methodologies of Amino Acid Analysis

The amino acid analysis techniques include the postcolumn derivatization for detection (Methods 1 to 2) after the separation of the free amino acids by ion-exchange chromatography and the precolumn derivatization of the free amino acids (Methods 2 to 7) followed by reversed-phase HPLC.

(i) Method l: Ninhydrin

(ii) Method 2: *o*-Phthalaldehyde (OPA)

(iii) Method 3: Phenylisothiocyanate (PITC)

(iv) Method 4: 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC)

(v) Method 5: (Dimethylamino)azobenzenesulfonyl chloride (DABS-Cl)

(vi) Method 6: 9-Fluorenylmethyl chloroformate (FMOC-Cl)

(vii) Method 7: 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F)

Among these methods, ion-exchange chromatography with postcolumn ninhydrin derivertization is one of the most common methods employed for quantitative amino acid analysis. The choice of any one technique often depends on the sensitivity required from the assay. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic sys-

tems, etc. Specific parameters may vary according to the exact equipment and procedure used.

3.01 Determination of Bulk and Tapped Densities

Change to read:

This determination is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

◆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.◆

Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/mL) although the international unit is kilogram per cubic meter (1 g/mL = 1000 kg/m^3) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm^3).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may have been passed through a screen, into a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or a measuring vessel (Method 3). Method 1 and Method 3 are favoured.

Method 1: Measurement in a Graduated Cylinder

Procedure

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 mL (readable to 2 mL), gently introduce, without compacting, approximately 100 g of the test sample (m) weighed with 0.1 per cent accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in g per mL by the formula m/V_0. Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample, such that its untapped apparent volume is 150 mL to 250 mL (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 mL and 100 mL a 100 mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Method 2: Measurement in a Volumeter

Apparatus

The apparatus(*1*) (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or a square (16.39 ± 2.00 mL volume with inside dimensions of 25.4 ± 0.076 mm).

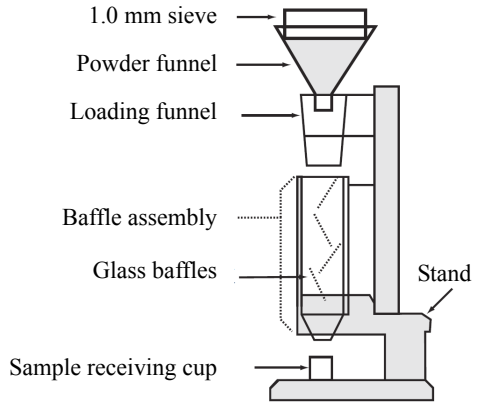


Fig. 3.01-1 Volumeter

Procedure

Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm^3 of powder with the square cup and 35 cm^3 of powder with the cylindrical cup. Carefully, scrape excess powder from the top of the cup by smoothly moving the edge of the blade of spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the side of the cup and determine the mass (m) of the powder to the nearest 0.1 per cent. Calculate the bulk density in

g per mL by the formula m/V_0 in which V_0 is the volume of the cup and record the average of 3 determinations using 3 different powder samples.

(I) The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.

Method 3: Measurement in a Vessel

Apparatus

The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Fig. 3.01-2.

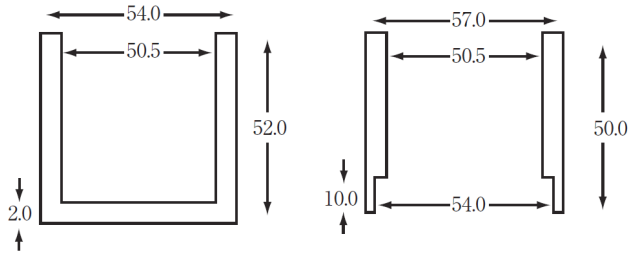


Fig. 3.01-2. Measuring vessel (left) and cap (right)
Dimensions in mm

Procedure

Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method 2. Determine the mass (m_0) of the powder to the nearest 0.1 per cent by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula $m_0/100$ and record the average of 3 determinations using 3 different powder samples.

Tapped Density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance by either of 3 methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

Method 1

Apparatus

The apparatus (Fig. 3.01-3) consists of the following:

– a 250 mL graduated cylinder (readable to 2 mL) with a mass of 220 ± 44 g.

– a settling apparatus capable of producing, in 1 min, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

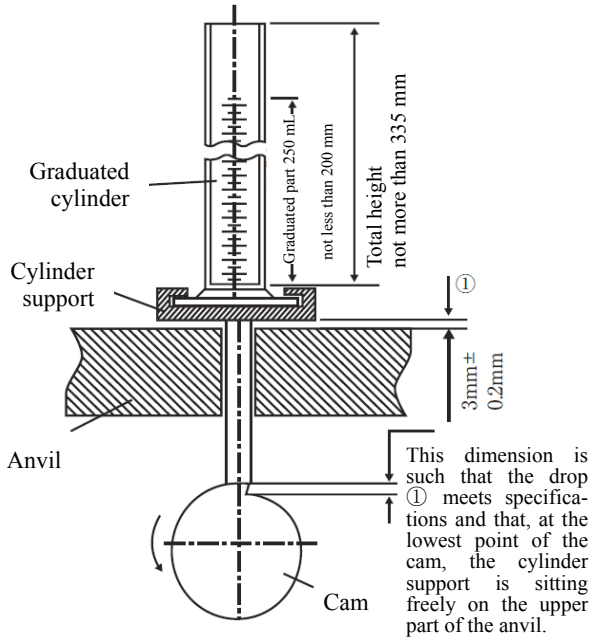


Fig. 3.01-3.

Procedure

Proceed as described above for the determination of the bulk volume (V_0).

Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500} and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_f in which V_f is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

Method 2

Procedure

Proceed as directed under Method 1 except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per minute.

Method 3

Procedure

Proceed as described in the method for measuring the bulk

density using the measuring vessel equipped with the cap shown in Fig. 3.01-2. The measuring vessel with the cap is lifted 50-60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrape excess powder from the top of the measuring vessel as described in Method 3 for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the 2 masses obtained after 200 and 400 taps exceeds 2 per cent, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2 per cent. Calculate the tapped density (g/mL) using the formula $m_f/100$ where m_f is the mass of powder in the measuring vessel. Record the average of 3 determinations using 3 different powder samples.

Measures of Powder Compressibility

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the Compressibility Index or the Hausner Ratio.

The Compressibility Index and Hausner Ratio are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio.

Compressibility Index:

$$100\ (V_0 - V_f)/V_0$$

V_0: unsettled apparent volume
V_f: final tapped volume

Hauser Ratio:

$$V_0/V_f$$

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0.

3.02 Specific Surface Area by Gas Adsorption

Change to read:

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

◆The specific surface area determination method is a method to determine specific surface area (the total surface area of powder per unit mass) of a pharmaceutical powder sample by using gas adsorption method.◆ The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

1.1 MULTI-POINT MEASUREMENT

When the gas is physically adsorbed by the powder sample, the following relationship (Brunauer, Emmett and Teller (BET) adsorption isotherm) holds when the relative pressure (P/P_0) is in the range of 0.05 to 0.30 for pressure P of the adsorbate gas in equilibrium for the volume of gas adsorbed, V_a.

$$1/[V_a\{(P_0/P) - 1\}] = \{(C - 1)/(V_mC)\} \times (P/P_0) + 1/(V_mC) \qquad (1)$$

P: Partial vapour pressure of adsorbate gas in equilibrium with the surface at -195.8ºC (b.p. of liquid nitrogen), in pascals,
P_0: Saturated pressure of adsorbate gas, in pascals,
V_a: Volume of gas adsorbed at standard temperature and pressure (STP) [0ºC and atmospheric pressure (1.013×10^5 Pa), in milliliters,
V_m: Volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters,
C: Dimensionless constant that is related to the enthalpy of adsorption of the adsorbate gas on the powder sample.

A value of V_a is measured at each of not less than 3 values of P/P_0. Then the BET value, $1/[V_a\{(P_0/P) - 1\}]$, is plotted against P/P_0 according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient, r, of the linear regression is not less than 0.9975; that is, r^2 is not less than 0.995. From the resulting linear plot, the slope, which is equal to $(C - 1)/(V_mC)$, and the intercept, which is equal to $1/(V_mC)$, are evaluated by linear regression analysis. From these values, V_m is calculated as 1/(*slope* + *intercept*), while C is calculated as (*slope*/*intercept*) + 1. From the value of V_m so determined, the specific surface area, S, in m^2g^{-1}, is calculated by the equation:

$$S = (V_mNa) / (m \times 22400) \qquad (2)$$

N: Avogadro constant (6.022×10^{23} mol^{-1}),
a: Effective cross-sectional area of one adsorbate molecule, in square metres (0.162×10^{-18} m^2 for nitrogen and 0.195×10^{-18} m^2 for krypton),
m: Mass of test powder, in grams,
22400: Volume, in milliliters, occupied by one mole of the adsorbate gas at STP allowing for minor departures from the ideal.

A minimum of 3 data points is required. Additional measurements may be carried out, especially when non-linearity is ob-

tained at a P/P_0 value close to 0.3. Because non-linearity is often obtained at a P/P_0 value below 0.05, values in this region are not recommended. The test for linearity, the treatment of the data, and the calculation of the specific surface area of the sample are described above.

1.2 SINGLE-POINT MEASUREMENT

Normally, at least 3 measurements of V_a each at different values of P/P_0 are required for the determination of specific surface area by the dynamic flow gas adsorption technique (*Method I*) or by volumetric gas adsorption (*Method II*). However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of V_a measured at a single value of P/P_0 such as 0.300 (corresponding to 0.300 mole of nitrogen or 0.001038 mole fraction of krypton), using the following equation for calculating V_m:

$$V_m = V_a\{1-(P/P_0)\} \quad (3)$$

The single-point method may be employed directly for a series of powder samples of a given material for which the material constant C is much greater than unity. These circumstances may be verified by comparing values of specific surface area determined by the single-point method with that determined by the multiple-point method for the series of powder samples. Close similarity between the single-point values and multiple-point values suggests that $1/C$ approaches zero. The single-point method may be employed indirectly for a series of very similar powder samples of a given material for which the material constant C is not infinite but may be assumed to be invariant. Under these circumstances, the error associated with the single-point method can be reduced or eliminated by using the multiple-point method to evaluate C for one of the samples of the series from the BET plot, from which C is calculated as (1 + *slope*/*intercept*). Then V_m is calculated from the single value of V_a measured at a single value of P/P_0 by the equation:

$$V_m = V_a\{(P_0/P) - 1\}[(1/C) + \{(C-1)/C\} \times (P/P_0)] \quad (4)$$

2. SAMPLE PREPARATION

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface during storage and handling. If outgassing is not achieved, the specific surface area may be reduced or may be variable because some parts of surface area are covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials. The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure and time should be so chosen that the original surface of the solid is reproduced as closely as possible.

Outgassing of many substances is often achieved by applying a vacuum, by purging the sample in a flowing stream of a non-reactive, dry gas, or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

The standard technique is the adsorption of nitrogen at liquid nitrogen temperature.

For powders of low specific surface area (<0.2 m^2g^{-1}) the proportion adsorbed is low. In such cases the use of krypton at liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error. All gases used must be free from moisture.

Accurately weigh a quantity of the test powder such that the total surface of the sample is at least 1 m^2 when the adsorbate is nitrogen and 0.5 m^2 when the adsorbate is krypton. Lower quantities of sample may be used after appropriate validation.

Because the amount of gas adsorbed under a given pressure tends to increase on decreasing the temperature, adsorption measurements are usually made at a low temperature. Measurement is performed at -195.8ºC, the boiling point of liquid nitrogen.

Adsorption of gas should be measured either by Method I or Method II.

3.1 Method I: the dynamic flow method

In the dynamic flow method (see Fig. 3.02-1), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions. A minimum of 3 mixtures of the appropriate adsorbate gas with helium are required within the P/P_0 range 0.05 to 0.30.

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of 3 data points within the recommended range of 0.05 to 0.30 for P/P_0 is to be determined.

A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again through the thermal conductivity cell and then to a recording potentiometer. Immerse the sample cell in liquid nitrogen, then the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

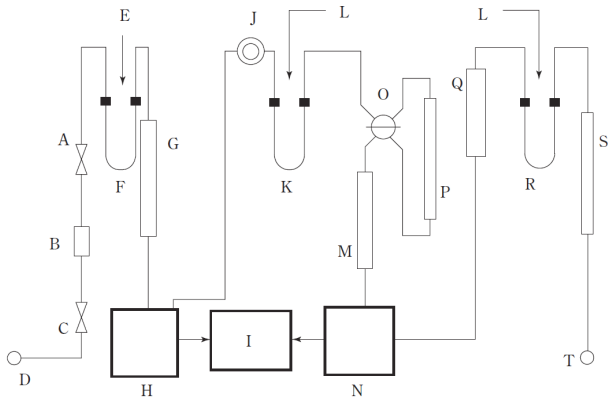
Remove from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak.

Since this is better defined than the adsorption peak, it is the one used for the determination.

To effect the calibration, inject a known quantity of adsorbate into the system, sufficient to give a peak of similar magnitude to

the desorption peak and obtain the proportion of gas volume per unit peak area.

Use a nitrogen/helium mixture for a single-point determination and several such mixtures or premixing 2 streams of gas for a multiple-point determination. Calculation is essentially the same as for the volumetric method.



A: Flow control valve
B: Differential flow controller
C: On-off valve
D: Gas inlet
E: O ring seals
F: Cold trap
G: Thermal equilibration tube
H: Detector
I: Digital display
J: Calibrating septum
K: Sample cell
L: Self seals quick connection
M: Short path ballast
N: Detector
O: Path selection valve
P: Long path ballast
Q: Flow meter
R: Outgassing station
S: Diffusion baffle
T: Vent

Fig. 3.02-1 Schematic diagram of the dynamic flow method apparatus

3.2 Method II: the volumetric method

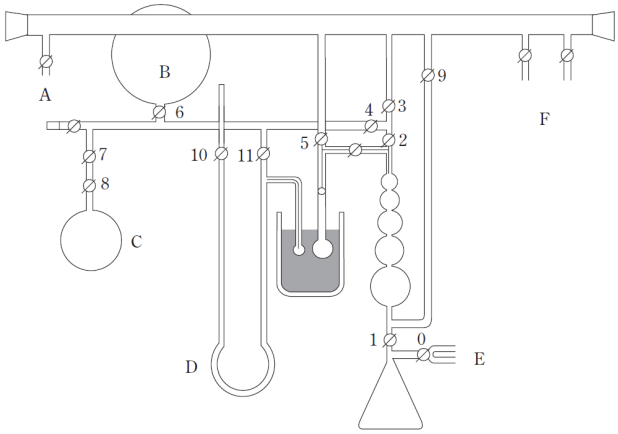
In the volumetric method (see Fig. 3.02-2), the recommended adsorbate gas is nitrogen which is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure, P, of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Since only pure adsorbate gas, instead of a gas mixture, is employed, interfering effects of thermal diffusion are avoided in this method.

Admit a small amount of dry nitrogen into the sample tube to prevent contamination of the clean surface, remove the sample tube, insert the stopper, and weigh it. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure (e.g. between 2 Pa and 10 Pa). Alternately, some instruments operate by evacuating to a defined rate of pressure change (e.g. less than 13 Pa/30 s) and holding for a defined period of time before commencing the next step.

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, for example, by the admission of a non-adsorbed gas, such as helium, this procedure is carried out at this point, followed by evacuation of the sample. The determination of dead volume may be avoided using difference measurements, that is, by means of reference and sample tubes connected by a differential transducer.

Raise a Dewar vessel containing liquid nitrogen at -195.8ºC up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed, V_a. For multipoint measurements, repeat the measurement of V_a at successively higher P/P_0 values. When nitrogen is used as the adsorbate gas, P/P_0 values of 0.10, 0.20, and 0.30 are often suitable.



A: Vacuum gauge
B: Nitrogen reservoir
C: Helium reservoir
D: Vapour pressure manometer
E: Vacuum air
F : To cold traps and vacuum pumps

Fig. 3.02-2 Schematic diagram of the volumetric method apparatus

4. REFERENCE MATERIALS

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as α-alumina for specific surface area determination, which should have a specific surface area similar to that of the sample to be examined.

3.03 Powder Particle Density Determination

Change to read:

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the test that are not harmonized are marked with symbols (◆ ◆).

Powder Particle Density Determination is ◆a method to determine particle density of powdered pharmaceutical drugs or raw materials of drugs◆, and the gas displacement pycnometer is generally used. The gas pycnometric density is determined by measuring the volume occupied by a known mass of powder which is equivalent to the volume of gas displaced by the powder using a gas displacement pycnometer. In gas pycnometric density measurements, the volume determined excludes the volume occupied by open pores; however, it includes the volume occupied by sealed pores or pores inaccessible to the gas.

Usually, helium is used as a test gas due to its high diffusivity into small open pores. If gases other than helium are used, different values would be obtained, since the penetration of the gas is dependent on the size of the pore as well as the cross-sectional area of the gas.

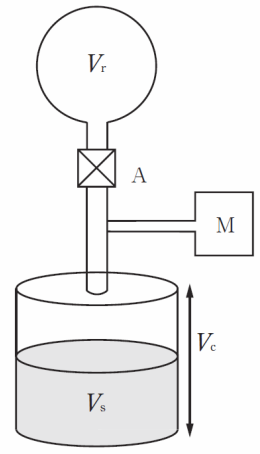
The measured density is a volume weighted average of the densities of individual powder particles. It is called the particle density, distinct from the true density of solid or the bulk density of powder. The density of solids are expressed in grams per cubic centimeter (g/cm^3), although the international unit is the kilogram per cubic meter (1 g/cm^3 = 1000 kg/m^3).

1. Apparatus

The schematic diagram of particle density apparatus for gas displacement pycnometric measurement is shown in Fig. 3.03-1. The apparatus consists of a test cell in which the sample is placed, an expansion cell and a manometer (M). The test cell, with an empty cell volume (V_c), is connected through a valve (A) to an expansion cell, with a volume (V_r).

Generally, helium is used as the measurement gas. The apparatus has to be equipped with a system capable of pressuring the test cell to the defined pressure (P) through the manometer.

Calibration of apparatus The volumes of the test cell (V_c) and the expansion cell (V_r) must be accurately determined to the nearest 0.001 cm^3, and to assure accuracy of the results of volume obtained, calibration of the apparatus is carried out as follows using a calibration ball of known volume for particle density measurement. The final pressures (P_f) are determined for the initial empty test cell followed by the test cell placed with the calibration ball for particle density measurement in accordance with the procedures, and V_c and V_r are calculated using the equation described in the section of Procedure. Calculation can be made taking into account that the sample volume (V_s) is zero in the first run.



A: Valve,
V_r: Expansion volume(cm^3)
V_c: Cell volume (cm^3)
V_s: Sample volume (cm^3)
M: Manometer

Fig. 3.03-1 Schematic diagram of a gas displacement pycnometer

2. Procedure

The gas pycnometric density measurement is performed at a temperature between 15ºC and 30ºC and must not vary by more than 2ºC during the course of measurement.

Volatile contaminants in the powder are removed by degassing the powder under a constant purge of helium prior to the measurement. Occasionally, powders may have to be degassed under vacuum. Because volatiles may be evolved during the measurement, weighing of the sample is done after the pycnometric measurement of volume.

Weigh the mass of the test cell and record it. After weighing out the amount of the sample as described in the individual monograph and placing it in the test cell, seal the cell in the pycnometer.

Open the valve (A) which connects the expansion cell with the test cell, confirm with the manometer (M) that the pressure inside the system is stable, and then read the system reference pressure (P_r). Secondly, close the valve that connects to the two cells, and introduce the measurement gas into the test cell to achieve positive pressure. Confirm with the manometer that the pressure inside the system is stable, and then read the initial pressure (P_i). Open the valve to connect the test cell with the expansion cell. After confirming that the indicator of the manometer is stable, read the final pressure (P_f), and calculate the sample volume (V_s) with the following equation.

$$V_s = V_c - \frac{V_r}{\dfrac{P_i - P_r}{P_f - P_r} - 1}$$

V_r : Expansion volume (cm^3)
V_c : Cell volume (cm^3)
V_s : Sample volume (cm^3)
P_i : Initial pressure (kPa)
P_f : Final pressure (kPa)
P_r : System reference pressure (kPa)

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume agree

to within 0.2%, and calculate the mean of sample volumes (V_s). Finally, unload the test cell, weigh the mass of the test cell, and calculate the final sample mass (m) by deducting the empty cell mass from the test cell mass. The powder particle density ρ is calculated by the following equation:

$$\rho = m/V_s$$

ρ: Powder particle density in g/cm^3,
m: Final sample mass in g,
V_s: Sample volume in cm^3

If the pycnometer differs in operation or construction from the one shown in Fig. 3.03-1, follow the instructions of the manufacturer of the pycnometer. The sample conditioning is indicated with the results. For example, indicate whether the sample was tested as is or dried under specific conditions such as those described for loss on drying.

3.04 Particle Size Determination

Change the Method 2 to read:

Method 2. Analytical Sieving Method

◆The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes. "Powder" here means a gathering of numerous solid particles.◆

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about 75 μm. For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than 75 μm where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

Estimate the particle size distribution as described under *Dry Sieving Method*, unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below 75 μm), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

Principles of Analytical Sieving—Analytical test sieves are constructed from a woven-wire mesh, which is of simple weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75 μm. The size parameter involved in determining particle size distribution by analytical sieving is the length of the size of the minimum square aperture through which the particle will pass.

TEST SIEVES

Test sieves suitable for pharmacopoeial tests conform to the most current edition of International Organisation for Standardization (ISO) Specification ISO 3310-1; Test sieves—Technical requirements and testing (see Table 3.04-1). Unless otherwise specified in the monograph, use those ISO sieves listed in the Table 3.04-1 as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a $\sqrt{2}$ progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Mesh numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable non-reactive wire.

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1[2)]. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated

optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 μm, Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

Cleaning Test Sieves—Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

Test Specimen—If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200 mm diameter. For 76 mm sieves the amount of material that can be accommodated is approximately $1/7^{th}$ that which can be accommodated on a 200 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller diameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

Agitation Methods—Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can induce either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

Endpoint Determination—The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

SIEVING METHODS

Mechanical agitation

Dry Sieving Method—Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and place the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. Reweigh each sieve, and determine the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint had been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, a different particle size analysis method should be used.

Air Entrainment Methods

Air Jet and Sonic Sifter Sieving—Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as *air jet* sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method*, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the *sonic sifting* method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air

that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic sifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 μm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

INTERPRETATION

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

[1] Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276.

[2] International Organization for Standardization (ISO) Specification ISO 3310-1; Test sieves-Technical requirements and testing

Table 3.04-1. Sizes of Standard Sieve Series in Range of Interest

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (microns)	European Sieve No.	Japan Sieve No.
Principal sizes	Supplementary sizes					
R 20/3	R 20	R 40/3				
11.20 mm	11.20 mm	11.20 mm			11200	
	10.00 mm					
		9.50 mm				
	9.00 mm					
8.00 mm	8.00 mm	8.00 mm				
	7.10 mm					
		6.70 mm				
	6.30 mm					
5.60 mm	5.60 mm	5.60 mm			5600	3.5
	5.00 mm					
		4.75 mm				4
	4.50 mm					
4.00 mm	4.00 mm	4.00 mm	5	4000	4000	4.7
	3.55 mm					
		3.35 mm	6			5.5
	3.15 mm					
2.80 mm	2.80 mm	2.80 mm	7	2800	2800	6.5
	2.50 mm					
		2.36 mm	8			7.5
	2.24 mm					
2.00 mm	2.00 mm	2.00 mm	10	2000	2000	8.6
	1.80 mm					
		1.70 mm	12			10
	1.60 mm					
1.40 mm	1.40 mm	1.40 mm	14	1400	1400	12
	1.25 mm					
		1.18 mm	16			14
	1.12 mm					
1.00 mm	1.00 mm	1.00 mm	18	1000	1000	16
	900 μm					
		850 μm	20			18
	800 μm					
710 μm	710 μm	710 μm	25	710	710	22
	630 μm					
		600 μm	30			26
	560 μm					
500 μm	500 μm	500 μm	35	500	500	30
	450 μm					
		425 μm	40			36
	400 μm					
355 μm	355 μm	355 μm	45	355	355	42
	315 μm					
		300 μm	50			50
	280 μm					
250 μm	250 μm	250 μm	60	250	250	60
	224 μm					
		212 μm	70			70
	200 μm					
180 μm	180 μm	180 μm	80	180	180	83
	160 μm					
		150 μm	100			100
	140 μm					
125 μm	125 μm	125 μm	120	125	125	119
	112 μm					
		106 μm	140			140
	100 μm					
90 μm	90 μm	90 μm	170	90	90	166
	80 μm					
		75 μm	200			200
	71 μm					
63 μm	63 μm	63 μm	230	63	63	235
	56 μm					
		53 μm	270			282
	50 μm					
45 μm	45 μm	45 μm	325	45	45	330
	40 μm					
		38 μm			38	391

7.02 Test Methods for Plastic Containers

Change (11) under 2. Polyvinyl chloride containers for aqueous injections to read:

2. Polyvinyl chloride containers for aqueous injections

(11) Vinyl chloride—Wash a cut piece of a container with water, wipe thoroughly with a filter paper, subdivide into pieces smaller than 5-mm square, and place 1.0 g of them in a 20-mL volumetric flask. Add about 10 mL of tetrahydrofuran for gas chromatography, dissolve by occasional shaking in a cold place, add tetrahydrofuran for gas chromatography, previously cooled in a methanol-dry ice bath, to make 20 mL while cooling in a methanol-dry ice bath, and use this solution as the sample solution. Perform the tests as directed under Gas Chromatography *<2.02>* according to the operating conditions 1 and 2, using 10 μL each of the sample solution and Standard Vinyl Chloride Solution. The peak height of vinyl chloride from the sample solution is not more than that from the Standard Vinyl Chloride Solution under at least one of the conditions.

Operating conditions 1—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and 2 to 3 m in length, packed with 150 to 180 μm siliceous earth for gas chromatography coated with 15% to 20% polyalkylene glycol monoether for gas chromatography.

Column temperature: A constant temperature of between 60ºC and 70ºC.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 1.5 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

Operating conditions 2—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with 150 to 180 μm porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore size: 0.06-0.08 μm; 100-200 m^2/g).

Column temperature: A constant temperature of about 120ºC.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 3 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

9.01 Reference Standards

***Change the following in (1) to read*:**

*A: Assay
D: Dissolution
I: Identification
P: Purity
U: Uniformity of dosage units

(1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately.

Reference Standard	Intended Use*
Amlodipine Besilate	I, U, A
Azathioprine	I, U, A
Clomifene Citrate	I, U, A
Diethylcarbamazine Citrate	U, A
Ethenzamide	I, A
Probenecid	I, U, D, A
Prochlorperazine Maleate	I, U, D, A
Warfarin Potassium	I, U, D, A

***Change the following in (2) to read*:**

(2) The reference standards which are prepared by National Institute of Infectious Diseases.

Reference Standard	Intended Use*
Amoxicillin	I, D, A
Cefalexin	U, D, A
Cefatrizine Propylene Glycolate	I, D, A
Cefixime	I, P, U, D, A
Cefroxadine	I, U, D, A
Cefteram Pivoxil Mesitylene Sulfonate	U, D, A
Faropenem Sodium	I, P, U, D, A
Griseofulvin	I, P, U, D, A
Minocycline Hydrochloride	I, P, U, D, A
Pivmecillinam Hydrochloride	I, P, U, A

Add the following to (1):

Reference Standard	Intended Use*
Aciclovir	I, A
Calcitonin (Salmon)	A
Danazol	I, A
Diflucortolone Valerate	I, A
Doxazosin Mesilate	I, A
Fludrocortisone Acetate	I, A
Flutamide	I, A
Gefarnate	I, A
D-glucuronolactone	A
Indapamide	I, U, D, A
Ipriflavone	I, A
Losartan Potassium	I, A
Pioglitazone Hydrochloride	I, A
Prazosin Hydrochloride	I, A
Probucol	I, A
Sevoflurane	I, A
Simvastatin	I, A
Tacrolimus	I, A
Teprenone	I, A
Tosufloxacin Tosilate	I, U, D, A
Troxipide	I, U, D, A

Add the following to (2):

Reference Standard	Intended Use*
Tazobactam	I, A

9.41 Reagents, Test Solutions

Change the following to read:

Benzoylmesaconine hydrochloride for thin-layer chromatography $C_{31}H_{43}NO_{10}\cdot HCl\cdot xH_2O$ White crystals or crystalline powder. Soluble in water and in ethanol (99.5) and sparingly soluble in methanol. Melting point: about 250ºC (with decomposition).

Absorbance <2.24> $E_{1cm}^{1\%}$ (230 nm): 217 - 231 (5 mg calculated on the anhydrous basis, methanol, 200 mL)

Purity Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around R_f 0.4 appears.

Bergenin for thin-layer chromatography $C_{14}H_{16}O_9\cdot xH_2O$ White crystals or crystalline powder. Slightly soluble in ethanol (99.5), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification Determine the absorption spectrum of a solution of bergenin for thin-layer chromatography in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 217 nm and 221 nm, and between 273 nm, and 277 nm, and a minimum between 241nm and 245 nm.

Purity Related substances—Dissolve 1.0 mg of bergenin for thin-layer chromatography in 1 mL of methanol. Perform the test with 20 μL of this solution as directed in the Identification under Mallotus Bark: no spot other than the principal spot at the R_f value of about 0.5 appears.

(*E*)-Capsaicin for thin-layer chromatography $C_{18}H_{27}NO_3$ White crystals, having a strong irritative odor. Very soluble in methanol, freely soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 65-70ºC.

Purity Related substances—Dissolve 20 mg of (*E*)-capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 standard solution as directed in the Identification under Capsicum: any spot other than the principal spot at the R_f value of about 0.5 from the sample solution is not more intense than the spot from the standard solution.

Add the following:

Acemetacin $C_{21}H_{18}ClNO_6$ [Same as the namesake monograph]

Acemetacin for assay $C_{21}H_{18}ClNO_6$ [Same as the monograph Acemetacin. When died, it contains no less than 99.5% of acemetacin ($C_{21}H_{18}ClNO_6$) meeting the following additional specifications.]

Purity Dissolve 40 mg of acemetacin for assay in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than acemetacin is not larger than 1/2 times the peak area of acemetacin obtained from the standard solution, and the total area of the peaks other than the peak of acemetacin is not larger than the peak area of acemetacin 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 under Acemetacin Tablets.

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

System Suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of acemetacin obtained from 10 μL of this solution is equivalent to 3 to 7% of that of acemetacin from the standard solution.

System performance: Dissolve 75 mg of acemetacin and 75

mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of a solution of hexyl parahydroxybenzoate in methanol (1 in 250), and add methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acemetacin, indometacin and hexyl parahydroxybenzoate are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and hexyl parahydroxybenzoate being not less than 3, 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 acemetacin is not more than 1.5%.

Acetic acid buffer solution containing 0.1% bovine serum albumin Dissolve 0.1 g of bovine serum albumin in sodium acetate trihydrate solution (1 in 100) to make exactly 100 mL, and adjust the pH to 4.0 with 1 mol/L hydrochloric acid TS.

Acetic acid-sodium acetate TS, pH 7.0 Dissolve 4.53 g of sodium acetate trihydrate in water to make 100 mL, and adjust the pH to 7.0 with diluted acetic acid (100) (1 in 50).

Achyranthes root for thin-layer chromatography A heat-dried, pulverized root of *Achyranthes fauriei* Leveillé et Vaniot (*Amaranthaceae*) meeting the following additional specifications.

Identification (1) To 2 g of pulverized achyranthes root for thin-layer chromatography add 10 mL of water, shake for 10 minutes, add 5 mL of 1-butanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 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 Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and 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 105ºC for 10 minutes: the standard solution shows a deep purplish red spot at around R_f 0.35, and the sample solution shows spots equivalent to those described below:

R_f value	Color and shape of the spot
Around 0	A weak spot, black
Around 0.1	A weak spot, strong purplish red
Around 0.2	A weak, tailing spot, strong purplish red
Around 0.25	A strong spot, deep purplish red
Around 0.35	A leading spot, deep purplish red
Around 0.45	A weak spot, dull yellow
Around 0.5	A weak spot, grayish purplish red
Around 0.75	A weak spot, grayish red
Around 0.9	A weak spot, dull red

(2) Perform the test as directed in the operating conditions under (1), except using a mixture of 1-propanol, ethyl acetate and water (4:4:3) as the developing solvent: the standard solution shows a deep purplish red spot at around R_f 0.45, and the sample solution shows spots equivalent to those described below:

R_f value	Color and shape of the spot
Around 0.25	A weak spot, strongly purplish red
Around 0.25 - 0.3	A leading spot or two strong spots, strongly purplish red
Around 0.35	A deep purplish red spot
Around 0.4	A weak spot, dull red
Around 0.42	A dark red spot
Around 0.45	A weak spot, grayish red
Around 0.65	A weak spot, dull greenish yellow
Around 0.7	A weak spot, grayish red
Around 0.85	A weak spot, grayish red
Around 0.95	A weak spot, dull yellow-red

Aconitum monoester alkaloids standard TS for component determination Weigh accurately about 20 mg of benzoylmesaconine hydrochloride for component determination (separately, determine the water content), about 10 mg of benzoylhypaconine hydrochloride for component determination (separately, determine the water content) and about 20 mg of 14-anisoylaconine hydrochloride for component determination (separately, determine the water content), dissolve in a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17) to make exactly 1000 mL. Perform the test with 20 μL of this solution as directed in the Purity under benzoylmesaconine hydrochloride for component determination: the peaks of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine appear with a peak area ratio of about 2:1:2.

Alcian blue 8 GX $C_{56}H_{68}Cl_{14}CuN_{16}S_4$ Dark blue-purple powder.

Alcian blue staining solution Dissolve 0.5 g of alcian blue 8 GX in 100 mL of diluted acetic acid (100) (3 in 100).

Allopurinol $C_5H_4N_4O$ [Same as the namesake monograph.]

Allopurinol for assay $C_5H_4N_4O$ [Same as the monograph Allopurinol. When dried, it contains not less than 99.0% of allopurinol ($C_5H_4N_4O$).]

Alkaline copper (II) TS Dissolve 20 g of anhydrous sodium carbonate in dilute sodium hydroxide TS to make 1000 mL, and use this solution as solution A. Dissolve 0.5 g of copper (II) sulfate pentahydrate in potassium sodium tartarate tetrahydrate solution (1 in 100) to make 100 mL, and use this solution as solution B. To 50 mL of solution A add 1 mL of solution B. Prepare before use.

Alkaline phosphatase Obtained from bovine small intestine, a white to grayish white or yellow-brown, freeze-dried powder having no odor.

Alkaline phosphatase contains not less than 1 unit per mg and no salts. One unit of alkaline phosphatase indicates an amount of the enzyme which produces 1 μmol of 4-nitrophenol in 1

minute at 37ºC and pH 9.8, from 4-nitrophenylphosphate ester used as the substrate.

Alkaline phosphatase TS Dissolve 0.1 g of alkaline phosphatase in 10 mL of boric acid-magnesium chloride buffer solution, pH 9.0. Prepare before use.

6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate $C_{14}H_{11}N_3O_4$ Prepared for amino acid analysis or biochemistry.

2-Aminobenzimidazole $C_7H_7N_3$ White to light yellow crystals or crystalline powder. Melting point: about 231ºC (with decomposition).

Amiodarone hydrochloride for assay $C_{25}H_{29}I_2NO_3 \cdot HCl$ [Same as the monograph Amiodarone Hydrochloride. When dried, it contains not less than 99.5% of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3 \cdot HCl$).]

14-Anisoylaconine hydrochloride for component determination $C_{33}H_{47}NO_{11} \cdot HCl \cdot xH_2O$ White crystalline powder or powder. Freely soluble in methanol, sparingly soluble in water and in ethanol (99.5). Melting point: about 210ºC (with decomposition).

Absorbance <2.24> $E^{1\%}_{1cm}$ (258 nm): 276 - 294 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of 14-anisoylaconine hydrochloride for component determination add exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principle spot at around R_f 0.5 appears.

(2) Related substances—Dissolve 5.0 mg of 14-anisoylaconine hydrochloride for component determination in 5 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* 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 the peak of 14-anisoylaconine obtained from the sample solution is not larger than the peak area of 14-anisoylaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 4 times as long as the retention time of 14-anisoylaconine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of 14-anisoylaconine obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of 14-anisoylaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution for component determination under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine 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 aconitum monoester alkaloids standard solution for component determination under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

Aprindine hydrochloride for assay $C_{22}H_{30}N_2 \cdot HCl$ [Same as the monograph Aprindine Hydrochloride. When dried, it contains not less than 99.5% of aprindine hydrochloride ($C_{22}H_{30}N_2 \cdot HCl$).]

Anode solution A for water determination Dissolve 100 g of diethanolamine in 900 mL of a mixture of methanol for water determination and chloroform for water determination (1:1), pass dried sulfur dioxide gas through this solution while cooling until the mass increase of the solution reaches 64 g. Then add 20 g of iodine, and add water until the color of the solution changes from brown to yellow. To 600 mL of this solution add 400 mL of chloroform for water determination.

(*E*)-Asarone $C_{12}H_{16}O_3$ White powder. Freely soluble in methanol and in ethanol (99.5) and practically insoluble in water. Melting point: about 60ºC

Identification Determine the infrared absorption spectrum of (*E*)-asarone as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, it exhibits absorption at the wave numbers of about 2990 cm^{-1}, 2940 cm^{-1}, 2830 cm^{-1}, 1609 cm^{-1}, 1519 cm^{-1}, 1469 cm^{-1}, 1203 cm^{-1}, 1030 cm^{-1}, 970 cm^{-1} and 860 cm^{-1}.

Purity Related substances—Dissolve 2 mg of (*E*)-asarone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* 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 the peak of (*E*)-asarone obtained from the sample solution is not larger than the peak area of (*E*)-asarone 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 Perilla Herb.

Time span of measurement: About 3 times as long as the retention time of (*E*)-asarone beginning after the solvent peak.

System suitability

System performance: Proceed as directed in the system suitability in the Component determination under Perilla Herb.

Azelastine hydrochloride for assay $C_{22}H_{24}ClN_3O \cdot HCl$

[Same as the monograph Azelastine Hydrochloride.]

Benz[*a*]anthracene $C_{18}H_{12}$ White to yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 158 - 163ºC.

Identification Perform the test with benz[*a*]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak (*m*/*z* 228) and a fragment ion peak (*m*/*z* 114).

Purity Related substances—Dissolve 3.0 mg of benz[*a*]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1 μL of this solution as directed under Gas Chromatography <*2.02*>, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benz[*a*]anthracene is not more than 2.0%.

Operating conditions

Detector: A mass spectrophotometer (EI)

Mass scan range:15.00 - 300.00

Time of measurement: 12 - 30 minutes

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 - 0.5 μm.

Column temperature: Inject at a constant temperature of about 45ºC, raise the temperature to 240ºC at a rate of 40ºC per minute, maintain at 240ºC for 5 minutes, raise to 300ºC at a rate of 4ºC per minute, raise to 320ºC at a rate of 10ºC pr minute, and maintain at 320ºC for 3 minutes.

Injection port temperature: At a constant temperature of about 250ºC.

Interface temperature: At a constant temperature of 300ºC.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of benz[*a*]anthracene is about 15 minutes.

Split ratio: Splitless

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of benz[*a*]anthracene obtained from 1 μL of this solution is equivalent to 5 to 15% of that of benz[*a*]anthracene from the standard solution.

Benzo[*a*]pyrene $C_{20}H_{12}$ Light yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 176 - 181ºC.

Identification Perform the test with benzo[*a*]pyrene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak (*m*/*z* 252) and a fragment ion peak (*m*/*z* 125).

Purity Related substances—Dissolve 3.0 mg of benzo[*a*]pyrene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1 μL of this solution as directed under Gas Chromatography <*2.02*> under the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than benzo[*a*]pyrene is not more than 3.0%.

Operating conditions

Detector: A mass spectrophotometer (EI)

Mass scan range: 15.00 - 300.00

Time of measurement: 12 - 30 minutes

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 - 0.5 μm.

Column temperature: Inject at a constant temperature of about 45ºC, raise the temperature to 240ºC at a rate of 40ºC per minute, maintain at 240ºC for 5 minutes, raise to 300ºC at a rate of 4ºC per minute, raise to 320ºC at a rate of 10ºC per minute, and maintain at 320ºC for 3 minutes.

Injection port temperature: A constant temperature of about 250ºC.

Interface temperature: A constant temperature of about 300ºC.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo[*a*]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL. Confirm that the peak area of benzo[*a*]pyrene obtained from 1 μL of this solution is equivalent to 5 to 15% of that of benzo[*a*]pyrene from the sample solution.

Benzoylhypaconine hydrochloride for component determination $C_{31}H_{43}NO_9 \cdot HCl \cdot xH_2O$ White crystals or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5). Melting point: about 230ºC (with decomposition).

Absorbance <*2.24*> $E^{1\%}_{1cm}$ (230 nm): 225 - 240 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of benzoylhypaconine hydrochloride for component determination add exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around R_f 0.5 appears.

(2) Related substance—Dissolve 5.0 mg of benzoylhypaconine hydrochloride for component determination in 5 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> 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 the peak of benzoylhypaconine obtained from the sample solution is not larger than the peak area of benzoylhypaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength:

245 nm).

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

System suitability

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

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution for component determination under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard solution for component determination under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

Benzoylmesaconine hydrochloride for component determination Benzoylmesaconine hydrochloride for thin-layer chromatography meeting the following additional specifications.

Purity Related substances—Dissolve 5.0 mg of benzoylmesaconine hydrochloride for component determination in 5 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* 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 the peak of benzoylmesaconine obtained from the sample solution is not larger than the peak area of benzoylmesaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 6 times as long as the retention time of benzoylmesaconine.

System suitability

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

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution for component determination under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum ester alkaloids standard solution for component determination under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

Bifonazole $C_{22}H_{18}N_2$ [Same as the namesake monograph.]

Bis(cis-3,3,5-trimethylcyclohexyl) phthalate
$C_4H_4[COOC_6H_8(CH_3)_3]_2$ White crystalline powder.

Melting point <2.60>: 91 - 94ºC

Bisdemethoxycurcumin $C_{19}H_{16}O_4$ Yellow to orange crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: 213 - 217ºC.

Identification Determine the absorption spectrum of a solution of bisdemethoxycurcumin in methanol (1 in 400000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 413 nm and 417 nm.

Purity Related substances (1) Dissolve 4 mg of bisdemethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at R_f value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of bisdemethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography *<2.01>* under the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of bisdemethoxycurcumin obtained from the sample solution is not larger than the peak area of bisdemethoxycurcumin from the standard solution.

Operating conditions

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

Detector: A visible absorption photometer (wavelength: 422 nm).

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

System suitability

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

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of bisdemethoxycurcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of bisdemethoxycurcumin from the standard solution.

Bis(1,1-trifluoroacetoxy)iodobenzene $C_{10}H_5F_6IO_4$ Prepared for amino acid analysis or biochemistry.

Blood agar medium Sterilize 950 mL of heart infusion agar medium under increased pressure. Allow the media to cool to about 50ºC, add 50 mL of horse or sheep defibrinated blood, dispense in sterilized Petri dishes, and make them as plate media.

1% blood suspension Wash a defibrinated animal blood in isotonic solution, and make it into suspension to contain 1 vol%. Prepare before use.

Boric acid-magnesium chloride buffer solution, pH 9.0 Dissolve 3.1 g of boric acid in 210 mL of dilute sodium hydroxide, and add 10 mL of magnesium chloride hexahydrate (1 in 50) and water to make 1000 mL. Adjust the pH to 9.0, if necessary.

Cadralazine for assay $C_{12}H_{21}N_5O_3$ [Same as the monograph Cadralazine. When dried, it contains not less than 99.0% of cadralazine ($C_{12}H_{21}N_5O_3$).]

Carbazole $C_{12}H_9N$ White to nearly white foliaceous or plate-like crystals or crystalline powder. Freely soluble in pyridine and in acetone, slightly soluble in ethanol (99.5), and practically insoluble in water. It readily sublimes when heated.

Melting point <*2.60*>: 243 - 245ºC

Purity Clarity and color of solution—To 0.5 g of carbazole add 20 mL of ethanol (99.5), and dissolve by warming: the solution is clear.

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

Carbazole TS Dissolve 0.125 g of carbazole in ethanol (99.5) to make 100 mL.

Chlorhexidine hydrochloride $C_{22}H_{30}Cl_2N_{10}\cdot 2HCl$ [Same as the monograph Chlorhexidine Hydrochloride.]

(2-Chloroethyl) diethylamine hydrochloride $C_6H_{14}ClN\cdot HCl$ White powder.

Content: not less than 95.0%. Assay—Weigh accurately about 0.2 g of (2-chloroethyl)diethylamine hydrochloride, previously dried at 45ºC for 3 hours under reduced pressure, and dissolve in 15 mL of acetic acid (100). To this solution add 10 mL of a mixture of acetic acid (100) and mercury (II) acetate TS for nonaqueous titration (5:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.21 mg of $C_6H_{14}ClN\cdot HCl$

Cinoxacin for assay $C_{12}H_{10}N_2O_5$ [Same as the monograph Cinoxacin. When dried, it contains not less than 99.0% of cinoxacin ($C_{12}H_{10}N_2O_5$)]

***p*-Cresol** C_7H_8O [K 8306, Special class]

Curcumin for component determination $C_{21}H_{20}O_6$ Yellow to orange crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

Absorbance <*2.24*> $E_{1cm}^{1\%}$ (422 nm): 1460 - 1700 (dried for 24 hours in a desiccator (in vacuum, silica gel), 2.5 mg, methanol, 1000 mL).

Melting point <*2.60*>: 180 - 184ºC.

Purity Related substances—(1) Dissolve 4 mg of curcumin for component determination in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the R_f value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of curcumin for component determination in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography <*2.01*>. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of curcumin obtained from the sample solution is not larger than the peak area of curcumin from the standard solution.

Operating conditions

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

Detector: A visible absorption photometer (wavelength: 422 nm).

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

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Turmeric.

Test for required detectability: Pipet 1 mL of the standard solution, add methanol to make exactly 20 mL. Confirm that the peak area of curcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of curcumin from 10 μL of the standard solution.

Cyanopropylmethylphenylsilicone for gas chromatogra-

phy Prepared for gas chromatography.

Demethoxycurcumin $C_{20}H_{18}O_5$ Yellow to orange crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 166 - 170ºC.

Identification Determine the absorption spectrum of a solution of demethoxycurcumin in methanol (1 in 400000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 416 nm and 420 nm.

Purity Related substances—(1) Dissolve 4 mg of demethoxycurcumin in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot at the R_f value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

(2) Dissolve 1.0 mg of demethoxycurcumin in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of demethoxycurcumin obtained from the sample solution is not larger than the peak area of demethoxycurcumin from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Component determination under Turmeric

Detector: A visible absorption photometer (wavelength: 422 nm)

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

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Turmeric.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of demethoxycurcumin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of demethoxycurcumin from the standard solution.

Dibenz[*a,h*]anthracene $C_{22}H_{14}$ Very pale yellow to green-yellow crystalline powder or powder. Practically insoluble in water, in methanol and in ethanol (99.5). Melting point: 265 - 270ºC.

Identification Perform the test with dibenz[*a,h*]anthracene as directed in the Purity: the mass spectrum of the main peak shows a molecular ion peak (*m*/*z* 278) and a fragment ion peak (*m*/*z* 139).

Purity Related substances—Dissolve 3.0 mg of dibenz[*a,h*]anthracene in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 1 μL of this solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than dibenz[*a,h*]anthracene is not more than 7.0%.

Operating conditions

Detector: A mass spectrophotometer (EI)

Mass scan range: 15.00 - 300.00

Time of measurement: 12 - 30 minutes

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 0.25 - 0.5 μm.

Column temperature: Inject at a constant temperature of about 45ºC, raise the temperature to 240ºC at a rate of 40ºC per minute, maintain at 240ºC for 5 minutes, raise to 300ºC at a rate of 4ºC per minute, raise to 320ºC at a rate of 10ºC per minute, and maintain at 320ºC for 3 minutes.

Injection port temperature: A constant temperature of about 250ºC.

Interface temperature: A constant temperature of about 300ºC.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the reaction time of the peak of dibenz[a,h]anthracene is about 27 minutes.

Split ratio: Splitless

System suitability

Test for required detectability: Pipet 1 mL of the sample solution, and add methanol to make exactly 10 mL. Confirm that the peak area of dibenz[*a,h*]anthracene obtained from 1 μL of this solution is equivalent to 5 to 15% of that of dibenz[*a,h*]anthracene from the standard solution.

Dibutylamine $C_8H_{19}N$ Colorless, clear liquid.

Refractive index <*2.45*> n_D^{20}: 1.415 - 1.419

Density <*2.56*> (20ºC): 0.756 - 0.761 g/mL

2,6-Dichloroindophenol sodium-sodium acetate TS Mix equal volumes of 2,6-dichloroindophenol sodium dihydrate solution (1 in 20) and acetic acid-sodium acetate TS, pH 7.0. Prepare before use.

Diisopropylamine $[(CH_3)_2CH]_2NH$ Colorless, clear liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline.

Refractive index <*2.45*> n_D^{20}: 1.391 - 1.394

Specific gravity <*2.56*> d_{20}^{20}: 0.715 - 0.722

Dimenhydrinate for assay $C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$ [Same as the monograph Dimenhydrinate. When dried, it contains not less than 53.8% and not more than 54.9% of diphen-

hydramine ($C_{17}H_{21}NO$) and not less than 45.2% and not more than 46.1% of 8-chlorotheophylline ($C_7H_7ClN_4O_2$).]

Dimethoxymethane $C_3H_8O_2$ Colorless, clear and volatile liquid. Miscible with methanol, with ethanol (95) and with diethyl ether.

(Dimethylamino)azobenzenesulfonyl chloride $C_{14}H_{14}ClN_3O_2S$ Prepared for amino acid analysis or biochemistry.

Disodium hydrogen phosphate-citric acid buffer solution, pH 7.5 Dissolve 5.25 g of citric acid monohydrate in water to make 1000 mL. Add this solution to 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS to adjust the pH to 7.5.

Dithiodiglycolic acid $C_4H_6O_4S_2$ Prepared for amino acid analysis or biochemistry.

Dithiodipropionic acid $C_6H_{10}O_4S_2$ Prepared for amino acid analysis or biochemistry.

Droxidopa for assay $C_9H_{11}NO_5$ [Same as the monograph Droxidopa. When dried, it contains not less than 99.5% of droxidopa ($C_9H_{11}NO_5$).]

Ecabet sodium hydrate for assay $C_{20}H_{27}NaO_5S \cdot 5H_2O$ [Same as the monograph Ecabet Sodium Hydrate. When dried, it contains not less than 99.5% of ecabet sodium ($C_{20}H_{27}NaO_5S$).]

Emorfazone for assay $C_{11}H_{17}N_3O_3$ [Same as the monograph Emorfazone. When dried, it contains not less than 99.0% of emorfazone ($C_{11}H_{17}N_3O_3$).]

9-Fluorenylmethyl chloroformate $C_{15}H_{11}ClO_2$ Prepared for amino acid analysis or biochemistry.

7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole $C_6H_2FN_3O_3$ Prepared for amino acid analysis or biochemistry.

Flutoprazepam for assay $C_{19}H_{16}ClFN_2O$ [Same as the monograph Flutoprazepam. When dried, it contains not less than 99.5% of flutoprazepam ($C_{19}H_{16}ClFN_2O$).]

Guaiacol for assay $C_7H_8O_2$ Colorless to yellow clear liquid or colorless crystals with a characteristic, aromatic odor. Miscible with methanol and with ethanol (99.5), and sparingly soluble in water. Congealing point: 25 - 30ºC.

Identification Determine the infrared absorption spectrum of guaiacol for assay as directed in the ATR method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 1595 cm^{-1}, 1497 cm^{-1}, 1443 cm^{-1}, 1358 cm^{-1}, 1255 cm^{-1}, 1205 cm^{-1}, 1108 cm^{-1}, 1037 cm^{-1}, 1020 cm^{-1}, 916 cm^{-1}, 833 cm^{-1}, and 738 cm^{-1}.

Purity Related substances—Perform the test with 0.5 μL of guaiacol for assay as directed under Gas Chromatography *<2.02>* according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of guaiacol for assay is not more than 2.0%

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polymethylsiloxane for gas chromatography in 0.25 to 0.5 μm in thickness.

Column temperature: Raise the temperature from 100ºC to 130ºC at a rate of 5ºC per minute, raise to 140ºC at a rate of 2ºC per minute, raise to 200ºC at a rate of 15ºC per minute, and maintain at 200ºC for 2 minutes.

Injection port temperature: 200ºC.

Detector temperature: 250ºC.

Carrier gas: Helium

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

Split ratio: 1:50

System suitability

Test for required detectability: Weigh accurately about 70 mg of guaiacol for assay, add methanol to make exactly 100 mL, and use this solution for the solution for system suitability test. Confirm that the peak area of guaiacol obtained from 1 μL of the solution for system suitability test is equivalent to 0.08 to 0.16% of that of guaiacol obtained when 0.5 μL of guaiacol for assay is injected.

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of guaiacol are not less than 200000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 2.0%.

4′-*O*-Glucosyl-5-*O*-methylvisamminol for thin-layer chromatography $C_{22}H_{28}O_{10}$ White crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water.

Identification Determine the absorption spectrum of a solution of 4′-*O*-glucosyl-5-*O*-methylvisamminol for thin-layer chromatography in ethanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 286 nm and 290 nm.

Purity Related substances—Dissolve 1 mg of 4′-*O*-glucosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol. Perform the test with 5 μL of this solution directed in the Identification under Saposhnikovia Root: no spots other than the principal spot at around R_f 0.3 appears.

Guanine $C_5H_5N_5O$ White to pale yellowish white powder.

Absorbance <2.24> Weigh accurately about 10 mg of guanine, dissolve in 20 mL of dilute sodium hydroxide TS, and add 2 mL of 1 mol/L hydrochloric acid TS and 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL. Determine the absorbances, $E^{1\%}_{1\%}$, of this solution at 248 nm and 273 nm: they are between 710 and 770, and between 460 and 500, respectively.

Loss on drying <*2.41*>: Not more than 1.5% (0.5 g, 105ºC, 4 hours).

Heart infusion agar medium Prepared for biochemical tests.

Heptyl parahydroxybenzoate $C_{14}H_{20}O_3$ White crystals or crystalline powder.

Melting point <*2.60*>: 45 - 50ºC.

Content: Not less than 98.0% Assay—Weigh accurately about 3.5 g of heptyl parahydroxybenzoate, dissolve in 50 mL of diluted *N*,*N*-dimethylformamide (4 in 5), and titrate <*2.50*> with 1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 236.3 mg of $C_{14}H_{20}O_3$

Hexyl parahydroxybenzoate $C_{13}H_{18}O_3$ White crystals or crystalline powder.

Melting point <*2.60*>: 49 - 53ºC

Content: Not less than 98.0%. Assay—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS
= 222.3 mg of $C_{13}H_{18}O_3$

Hyodeoxycholic acid for thin-layer chromatography $C_{24}H_{40}O_4$ White to pale brown crystalline powder or powder. Freely soluble in methanol an in ethanol (99.5), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of hyodeoxycholic acid for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2940 cm^{-1}, 2840 cm^{-1}, 2360 cm^{-1}, 1740 cm^{-1}, 1460 cm^{-1}, 1340 cm^{-1}, 1200 cm^{-1}, 1160 cm^{-1}, 1040 cm^{-1} and 600 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +7 - +10° (0.4 g, ethanol (99.5), 20 mL, 100 mm).

Melting point <*2.60*>: 198 - 205ºC

Purity Related substances—Dissolve 20 mg of hyodeoxycholic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly dilute sulfuric acid on the plate, and heat at 105ºC for 10 minutes: the spots other than the principal spot at the R_f value of about 0.3 obtained from the sample solution are not more intense than the spot from the standard solution.

10-Hydroxy-2-(*E*)-decenoic acid for component determination 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography meeting the following additional specifications.

Purity Related substances—Dissolve 10 mg of 10-hydroxy-2-(*E*)-decenoic acid for component determination in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of 10-hydroxy-2-(*E*)-decenoic acid is not larger than the peak area of 10-hydroxy-2-(*E*)-decenoic acid 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 Royal Jelly.

Time span of measurement: About 4 times as long as the retention time of 10-hydroxy-2-(*E*)-decenoic acid beginning after the solvent peak.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Royal Jelly.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of 10-hydroxy-2-(*E*)-decenoic acid obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of 10-hydroxy-2-(*E*)-decenoic acid from the standard solution.

10-Hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography $C_{10}H_{18}O_3$ White crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), soluble in diethyl ether, and slightly soluble in water.

Identification Determine the absorption spectrum of a solution of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in ethanol (99.5) (1 in 125000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 206 nm and 210 nm.

Melting point <*2.60*>: 63 - 66ºC.

Purity Related substances—Dissolve 5.0 mg of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in 1 mL of diethyl ether. Perform the test with 20 μL of this solution as directed in the Identification under Royal Jelly: no spot other than the principal spot at around R_f 0.5 appears.

Imidapril hydrochloride $C_{20}H_{27}N_3O_6 \cdot HCl$ [Same as the monograph Imidapril Hydrochloride.]

Imidapril hydrochloride for assay $C_{20}H_{27}N_3O_6 \cdot HCl$ [Same as the monograph Imidapril Hydrochloride. When dried, it contains not less than 99.0% of imidapril hydrochloride ($C_{20}H_{27}N_3O_6 \cdot HCl$).]

Irsogladine maleate $C_9H_7Cl_2N_5 \cdot C_4H_4O_4$ [Same as the monograph Irsogladine Maleate.]

Irsogladine maleate for assay $C_9H_7Cl_2N_5 \cdot C_4H_4O_4$ [Same

as the monograph Irsogladine Maleate. When dried, it contains not less than 99.5% of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$).]

Isosorbide dinitrate for assay $C_6H_8N_2O_8$ [Same as the monograph Isosorbide Dinitrate. It contains not less than 99.0% of isosorbide dinitrate ($C_6H_8N_2O_8$) meeting the following additional specifications.]

Purity Related substances—Dissolve 50 mg of isosorbide dinitrate for assay in 50 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) 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 standard solution as directed under Liquid Chromatography *<2.01>*, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of isosorbide dinitrate obtained from the sample solution is not larger than the peak area of isosorbide dinitrate from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating procedures in the Assay under Isosorbide Dinitrate Tablets.

Time span of measurement: About 2 times as long as the retention time of isosorbide dinitrate beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 50 mL. Confirm that the peak area of isosorbide dinitrate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of isosorbide dinitrate from 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 factor of the peak of isosorbide dinitrate are not less than 3000 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 isosorbide dinitrate is not more than 2.0%.

Ketoconazole $C_{26}H_{28}Cl_2N_4O_4$ [Same as the namesake monograph.]

Ketoconazole for assay $C_{26}H_{28}Cl_2N_4O_4$ [Same as the monograph Ketoconazole. When dried, it contains not less than 99.5% of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$).]

4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde, mix gently, add gently 0.5 mL of sulfuric acid and 0.1 mL of acetic acid (100) in this order, and mix well.

Loganin for component determination Loganin for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1cm}^{1\%}$ (235 nm): 275 - 303 (dried in a desiccator (silica gel) for 24 hours, 5 mg, methanol, 500 mL).

Purity Related substances—Dissolve 2 mg of loganin for component determination in 5 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* 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 the peak of loganin is not larger than the peak area of loganin 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 (1) under Goshajinkigan Extract.

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

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of loganin obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of loganin from the standard solution.

Lovastatin $C_{24}H_{36}O_5$ White crystals or crystalline powder. Soluble in acetonitrile and in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Optical rotation <2.49> $[\alpha]_D^{20}$: +325 - +340° (50 mg calculated on the anhydrous basis, acetonitrile, 10 mL, 100 mm).

Loss on drying <2.41>: Not more than 1.0% (1 g, under reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Mebendazole $C_{16}H_{13}N_3O_3$ White powder. Practically insoluble in water and in ethanol (95).

Mercaptoethanesulfonic acid $C_2H_6O_3S_2$ Prepared for amino acid analysis or biochemistry.

2-Methoxy-4-methylphenol $C_8H_{10}O_2$ Colorless to pale yellow liquid. Miscible with methanol and with ethanol (99.5), and slightly soluble in water. Congealing point: 3 - 8°C.

Identification Determine the infrared absorption spectrum of 2-methoxy-4-methylphenol as directed in the ATR method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 1511 cm^{-1}, 1423 cm^{-1}, 1361 cm^{-1}, 1268 cm^{-1}, 1231 cm^{-1}, 1202 cm^{-1}, 1148 cm^{-1}, 1120 cm^{-1}, 1031 cm^{-1}, 919 cm^{-1}, 807 cm^{-1} and 788 cm^{-1}.

Purity Related substances—Perform the test with 0.2 μL of 2-methoxy-4-methylphenol as directed under Gas Chromatography *<2.02>* according to the following conditions. Determine each peak by the automatic integration method: the total area of the peaks other than the peak of 2-methoxy-4-methylphenol is

not more than 3.0%.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 60 m in length, coated inside with polymethylsiloxane for gas chromatography in 0.25 to 0.5 μm in thickness.

Column temperature: Inject at a constant temperature of about 100ºC, raise the temperature to 130ºC at a rate of 5ºC per minute, raise to 140ºC at a rate of 2ºC per minute, raise to 200ºC at a rate of 15ºC per minute, and maintain at 200ºC for 2 minutes.

Injection port temperature: 200ºC.

Detector temperature: 250ºC.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 2-methoxy-4-methylphenol is about 10 minutes.

Split ratio: 1:50

System suitability

System performance: Dissolve 60 mg of 2-methoxy-4-methylphenol in methanol to make 100 mL, and use this solution as the solution for system suitability test. Proceed with 1 μL of the solution for system suitability test under the above operating conditions: the symmetry factor of the peak of 2-methoxy-4-methylphenol is not more 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 peak area of 2-methoxy-4-methylphenol is not more than 2.0%.

Methyl acetate CH_3COOCH_3 [K 8382, Special class]

Mosapride citrate for assay $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7 \cdot 2H_2O$ [Same as the monograph Mosapride Citrate Hydrate. It contains not less than 99.0% of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) calculated on the anhydrous basis.]

Myristicin for thin-layer chromatography $C_{11}H_{12}O_3$ Colorless, clear liquid, having a characteristic odor. Miscible with ethanol (95), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of myristicin for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 3080 cm^{-1}, 2890 cm^{-1}, 1633 cm^{-1}, 1508 cm^{-1}, 1357 cm^{-1}, 1318 cm^{-1}, 1239 cm^{-1}, 1194 cm^{-1}, 1044 cm^{-1}, 994 cm^{-1}, 918 cm^{-1}, 828 cm^{-1} and 806 cm^{-1}.

Purity Related substances—Dissolve 20 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 0.5 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification under Nutmeg: the spots other than the principle spot at the R_f value of about 0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

Ofloxacin $C_{18}H_{20}FN_3O_4$ [Same as the namesake monograph.]

Perillaldehyde for component determination Perillaldehyde for thin-layer chromatography meeting the following specifications.

Absorbance <2.24> $E_{1cm}^{1\%}$ (230 nm): 850 - 950 (10 mg, methanol, 2000 mL).

Purity Related substances—Dissolve 10 mg of perillaldehyde in 250 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of perillaldehyde is not larger than the peak of perillaldehyde 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 Perilla Herb.

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

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under Perilla Herb.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of perillaldehyde obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of perillaldehyde from the standard solution.

Perillaldehyde for thin-layer chromatography $C_{10}H_{14}O$ Colorless to light brown transparent liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and very slightly soluble in water.

Identification Determine the infrared absorption spectrum of perillaldehyde for thin-layer chromatography as directed in the liquid film method under Infrared Spectrophotometry *<2.25>*: it exhibits absorption at the wave numbers of about 3080 cm^{-1}, 2930 cm^{-1}, 1685 cm^{-1}, 1644 cm^{-1}, 1435 cm^{-1} and 890 cm^{-1}.

Purity Related substances—Dissolve 1.0 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and perform the test with 10 μL of this solution as directed in the Identification under Perilla Herb: no spot other than the principal spot at around R_f 0.5 appears.

Phenyl isothiocyanate C_7H_5NS Prepared for amino acid analysis or biochemistry.

Phenobarbital for assay $C_{12}H_{12}N_2O_3$ [Same as the monograph Phenobarbital.]

Phenytoin for assay $C_{15}H_{12}N_2O_2$ [Same as the monograph Phenytoin meeting the following additional specifications.]

Purity Related substances—Dissolve 25 mg of phenytoin

for assay 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 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>. Determine each peak from both solutions by the automatic integration method: the total area of the peaks other than the peak of phenytoin obtained from the sample solution is not larger than the peak area of phenytoin from the standard solution.

Operating conditions

Column, column temperature and flow rate: Proceed as directed in the operating conditions in the Assay under Phenytoin Tablets.

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

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.5, and acetonitrile for liquid chromatography (11:9).

Time span of measurement: About 5 times as long as the retention time of phenytoin beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of phenytoin obtained from 10 μL of this solution is equivalent to 8 to 12% of that of phenytoin from 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 factor of the peak of phenytoin are not less than 6000 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 phenytoin is not more than 2.0%.

Plantago seed for thin-layer chromatography [Same as the monograph Plantago Seed meeting the following additional specifications.]

Identification (1) To 1 g of pulverized plantago seed for thin-layer chromatography add 3 mL of methanol, and warm on a water bath for 3 minutes. After cooling, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 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, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Splay evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105ºC for 10 minutes: spots equivalent to those described below appear.

R_f value	Color and shape of the spot
Around 0	A strong spot, very dark blue
Around 0.08	A very dark blue spot
Around 0.1 - 0.2	A leading spot, very dark blue
Around 0.25	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.35	A strong spot, dark grayish blue (corresponding to geniposidic acid)
Around 0.45	A weak spot, grayish yellowish green
Around 0.50	A strong spot, deep yellow-green (corresponding to acteoside)
Around 0.6	A weak spot, light blue
Around 0.85	A deep blue spot
Around 0.9 - 0.95	A tailing spot, grayish blue

(2) Proceed as directed in the operating conditions under (1), except using a mixture of ethyl acetate, water and formic acid (6:1:1) as developing solvent: spots equivalent to those described below appear.

R_f value	Color and shape of the spot
Around 0	A yellow-greenish dark gray spot
Around 0.05	A weak spot, dark grayish yellow-green
Around 0.2	A weak spot, dark green
Around 0.25	A strong spot, dark reddish purple (corresponding to geniposidic acid)
Around 0.35	A weak spot, bright blue
Around 0.4 - 0.45	A weak tailing spot, dull greenish blue
Around 0.45	A strong spot, deep yellow-green (corresponding to acteoside)
Around 0.5	A strong spot, deep blue (corresponding to plantagoguanidinic acid)
Around 0.95	A strong spot, dark grayish blue-green
Around 0.97	A dark grayish blue-green spot

Polyethylene glycol 2-nitroterephthalate for gas chromatography Prepared for gas chromatography.

Polymethylsiloxane for gas chromatography Prepared for gas chromatography.

(*E*)-2-methoxycinnamaldehyde for thin-layer chromatography $C_{10}H_{10}O_2$ White to yellow crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 44 - 50ºC.

Identification (1) Determine the absorption spectrum of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 282 nm and 286 nm, and between 331 nm and 335 nm.

(2) Determine the infrared absorption spectrum of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1675 cm^{-1}, 1620 cm^{-1}, 1490 cm^{-1}, 1470 cm^{-1}, 1295 cm^{-1}, 1165 cm^{-1}, 1130 cm^{-1}, 1025 cm^{-1} and 600 cm^{-1}.

Purity Related substances—Dissolve 10 mg of

(*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification (5) (ii) under Goshajinkigan Extract: the spots other than the principal spot at the R_f value of about 0.4 obtained from the sample solution are not more intense than the spot from the standard solution.

0.01 mol/L Potassium dihydrogen phosphate TS, pH 4.0 Dissolve 1.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Pig bile powder for thin-layer chromatography A yellow-gray to yellow-brown powder, having a characteristic odor and a bitter taste. It is practically insoluble in water, in methanol and in ethanol (99.5)

Identification To 0.1 g of pig bile powder for thin-layer chromatography in a screw-capped test tube, add 1 mL of sodium hydroxide solution (3 in 25), and shake. Heat the tube in an oil bath at 120ºC for 4 hours, allow to cool to a lukewarm temperature, add 2 mL of 3 mol/L hydrochloric acid TS and 2 mL of ethyl acetate, shake at 50ºC for 30 minutes, and separate ethyl acetate layer as the sample solution. Separately, dissolve 10 mg of hyodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) 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 105ºC for 10 minutes: one of the several spots obtained from the sample solution shows the same color tone and the same R_f value as the spot from the standard solution.

Propafenone hydrochloride for assay $C_{21}H_{27}NO_3 \cdot HCl$ [Same as the monograph Propafenone Hydrochloride. When dried, it contains not less than 99.0% of propafenone hydrochloride ($C_{21}H_{27}NO_3 \cdot HCl$). When proceed as directed in the Purity (2), the total area of the peaks other than the peak of propafenone is not larger than 3 times the peak area of propafenone from the standard solution.]

0.2 mol/L Pyridine-formic acid buffer solution, pH 3.0 To 15.82 g of pyridine add 900 mL of water, shake well, adjust the pH to 3.0 with diluted formic acid (1 in 2), and add water to make 1000 mL.

Rebamipide for assay $C_{19}H_{15}ClN_2O_4$ [Same as the monograph Rebamipide. When dried, it contains not less than 99.5% of rebamipide ($C_{19}H_{15}ClN_2O_4$).]

Sodium Azide NaN_3 [K 9501, Special class]

0.2 mol/L Sodium chloride TS Dissolve 11.7 g of sodium chloride in water to make 1000 mL.

Sodium glycocholate for thin-layer chromatography $C_{26}H_{42}NNaO_6 \cdot xH_2O$ White to pale brown crystalline powder or powder. Freely soluble in water and in methanol, and slightly soluble in ethanol (99.5). Melting point: about 260ºC (with decomposition).

Identification Determine the infrared absorption spectrum of sodium glycocholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, it exhibits absorption at the wave numbers of about 2940 cm^{-1}, 1640 cm^{-1}, 1545 cm^{-1}, 1450 cm^{-1}, 1210 cm^{-1}, 1050 cm^{-1}, and 600 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +25 - +35º (60 mg, methanol, 20 mL, 100 mm).

Purity Related substances—Dissolve 5 mg of sodium glycocholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 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 Thin-layer Chromatography <*2.03*>. Proceed with 5 μL each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot at the R_f value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

Sodium 1-nonanesulfonate $CH_3(CH_2)_8SO_3Na$ White crystalline powder. Freely soluble in water.

Loss on drying <*2.41*>: Not more than 1.0% (1 g, 105ºC, 3 hours).

Residue on ignition <*2.44*>: 30 - 32% (0.5 g).

Sodium tauroursodeoxycholate for thin-layer chromatography $C_{26}H_{44}NNaO_6S \cdot xH_2O$ White to pale brown crystalline powder or powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of sodium tauroursodeoxycholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits the absorption at the wave numbers of about 2940 cm^{-1}, 1600 cm^{-1}, 1410 cm^{-1}, 1305 cm^{-1}, 1195 cm^{-1}, 1080 cm^{-1}, 1045 cm^{-1}, 980 cm^{-1}, 950 cm^{-1}, 910 cm^{-1} and 860 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +40 - +50º (40 mg, methanol, 20 mL, 100 mm)

Purity Related substances—Dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 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 Thin-layer Chromatography <*2.03*>. Perform the test as directed in the Identification under Bear Bile: the spots other than the principal spot at the R_f value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

Sodium tetraborate-sulfuric acid TS To 9.5 g of sodium tetraborate decahydrate add 1000 mL of purified sulfuric acid,

and dissolve by shaking for a night.

Sodium valproate for assay $C_8H_{15}NaO_2$ [Same as the monograph Sodium Valproate. When dried, it contains not less than 99.0% of sodium valproate ($C_8H_{15}NaO_2$).]

Strontium TS Dissolve 76.5 g of strontium chloride in water to make exactly 500 mL. Pipet 20 mL of this solution, and add water to make exactly 1000 mL (1000 ppm).

Testosterone $C_{19}H_{28}O_2$ White crystals or crystalline powder.

Identification Determine the infrared absorption spectrum of testosterone as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*: it exhibits the absorption at the wave numbers of about 3530 cm^{-1}, 3380 cm^{-1}, 1612 cm^{-1}, 1233 cm^{-1}, 1067 cm^{-1} and 1056 cm^{-1}.

Theophylline for assay $C_7H_8N_4O_2$ [Same as the monograph Theophylline meeting the following additional specifications.]

Purity Related substances—Dissolve 50 mg of theophylline for assay in water to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>*. Determine each peak from both solutions by the automatic integration method: the total area of peaks other than the peak of theophylline obtained from the sample solution is not larger than the peak area of theophylline from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 270 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 40ºC.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

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

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

System suitability

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of theophylline obtained from 20 μL of this solution is equivalent to 15 to 25% of that of theophylline from 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 factor of the peak of theophylline are not less than 3000 and not more than 1.5, 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 theophylline is not more than 3.0%.

Tiapride hydrochloride for assay $C_{15}H_{24}N_2O_4S{\cdot}HCl$ [Same as the monograph Tiapride Hydrochloride.]

1% Triethylamine-phosphate buffer solution, pH 3.0 Dissolve 10 g of triethylamine in 950 mL of water, adjust the pH to 3.0 with phosphoric acid, and make exactly 1000 mL.

Tris(4-*t*-butylphenyl)phosphate $[(CH_3)_3CC_6H_4O]_3PO$ White crystals or crystalline powder.

Melting point <2.60>: 100 - 104ºC

Ubenimex for assay $C_{16}H_{24}N_2O_4$ [Same as the monograph Ubenimex. When dried, it contains not less than 99.0% of ubenimex ($C_{16}H_{24}N_2O_4$).]

Ursodeoxycholic acid for assay $C_{24}H_{40}O_4$ [Same as the monograph Ursodeoxycholic Acid. However, when dried, it contains not less than 99.0% of ursodeoxycholic acid ($C_{24}H_{40}O_4$) meeting the following additional specifications.]

Purity Related substances—Dissolve 0.15 g of ursodeoxycholic acid in 5 mL of methanol for liquid chromatography, and use this solution as the sample solution. Pipet 2 mL of this solution and add methanol for liquid chromatography to make exactly 50 mL. Pipet 2.5 mL of this solution, add methanol for liquid chromatography to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.5 with respect to ursodeoxycholic acid, obtained from the sample solution is not larger than the peak area of ursodeoxycholic acid from the standard solution, and the area of the peak, having the relative retention time of about 5.5, obtained from the sample solution is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution. Furthermore, the total area of the peaks other than the peak of ursodeoxycholic acid and other than the peaks mentioned above is not larger than 1/5 times the peak area of ursodeoxycholic acid from the standard solution.

Operating conditions

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

Column: A stainless steel column 3 mm in inside diameter and 7.5 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: A mixture of methanol for liquid chromatography, diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (96:69:35).

Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 2.3 minutes.

Time span of measurement: About 7 times as long as the retention time of ursodeoxycholic acid.

System suitability—

Test for required detectability: Pipet 2 mL of the standard so-

lution, and add methanol for liquid chromatography to make exactly 20 mL. Confirm that the peak area of ursodeoxycholic acid obtained from 5 μL of this solution is equivalent to 8 to 12% of that of ursodeoxycholic acid from the standard solution.

System performance: To 30 mg of chenodeoxycholic acid for thin-layer chromatography and 30 mg of lithocholic acid for thin-layer chromatography, add 1 mL of the sample solution, dissolve in methanol for liquid chromatography to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid are eluted in this order with the resolution between these peaks being not less than 7, respectively.

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

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

Sulfonamide group bound to hexadecylsilanized silica gel for liquid chromatography Prepared for column chromatography.

Official Monographs

Add the following:

Acemetacin Capsules

アセメタシンカプセル

Acemetacin Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$: 415.82).

Method of preparation Prepare as directed under Capsules, with Acemetacin.

Identification To an amount of powdered contents of Acemetacin Capsules, equivalent to 0.1 g of Acemetacin according to the labeled amount, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distil the methanol under reduced pressure. To the residue add 1 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same R_f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Acemetacin Capsules, add 40 mL of methanol, shake well, and add methanol to make exactly V mL so that each mL contains about 0.6 mg of acemetacin ($C_{21}H_{18}ClNO_6$). Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= W_S \times (Q_T/Q_S) \times (V/50)$

W_S: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 1000)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Acemetacin Capsules is not less than 70%.

Start the test with 1 capsule of Acemetacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 33 μg of acemetacin ($C_{21}H_{18}ClNO_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105ºC for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$)
$= W_S \times (A_T / A_S) \times (V'/V) \times (1/C) \times 180$

W_S: Amount (mg) of acemetacin for assay
C: Labeled amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$) in 1 capsule

Assay Take out the contents of not less than 20 Acemetacin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of acemetacin ($C_{21}H_{18}ClNO_6$), add 40 mL of methanol, shake well, and add methanol to make exactly 50 mL. Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105ºC for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acemetacin to that of the internal standard.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (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 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of 1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of acemetacin is about 7 minutes.

System suitability—

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 2 mL of this solution add 2 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, acemetacin, indometacin and the internal standard are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and the internal standard being not less than 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 ratio of the peak area of acemetacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Acemetacin Tablets

アセメタシン錠

Acemetacin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$: 415.82).

Method of preparation Prepare as directed under Tablets, with Acemetacin.

Identification To a quantity of powdered Acemetacin Tablets, equivalent to 0.1 g of Acemetacin according to the labeled amount, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distil the methanol under reduced pressure. Dissolve the residue in 1 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same R_f value.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Acemetacin Tablets add 3 mL of water, and shake until the tablet is disintegrated. Add 15 mL of methanol, shake for 20 minutes, and add methanol to make exactly V mL so that each mL contains about 1.2 mg of acemetacin ($C_{21}H_{18}ClNO_6$). Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of acemetacin } (C_{21}H_{18}ClNO_6) = W_S \times (Q_T/Q_S) \times (V/25)$$

W_S: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250)

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Acemetacin Tablets is not less than 80%.

Start the test with 1 tablet of Acemetacin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 33 μg of acemetacin ($C_{21}H_{18}ClNO_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105ºC for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

$$\text{Dissolution rate (\%) with respect to the labeled amount of acemetacin } (C_{21}H_{18}ClNO_6) = W_S \times (A_T / A_S) \times (V'/V) \times (1/C) \times 180$$

W_S: Amount (mg) of acemetacin for assay

C: Labeled amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Acemetacin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.6 g of acemetacin ($C_{21}H_{18}ClNO_6$), add 120 mL of methanol, shake for 20 minutes, and add methanol to make exactly 200 mL. Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly 1 mL

of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105ºC for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acemetacin to that of the internal standard.

$$\text{Amount (mg) of acemetacin } (C_{21}H_{18}ClNO_6) = W_S \times (Q_T/Q_S) \times 20$$

W_S: Amount (mg) of acemetacin for assay

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250)

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 40ºC.

Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of 1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of acemetacin is about 7 minutes.

System suitability—

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acemetacin, indometacin and the internal standard are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and the internal standard being not less than 3, respectively.

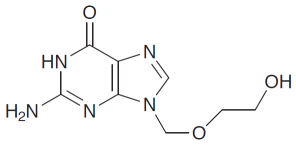
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acemetacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Aciclovir

アシクロビル



$C_8H_{11}N_5O_3$: 225.20

2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one [*59277-89-3*]

Aciclovir contains not less than 98.5 and not more than 101.0% of $C_8H_{11}N_5O_3$, calculated on the anhydrous basis.

Description Aciclovir occurs as a white to pale yellowish white crystalline powder.

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

It dissolves in 0.1 mol/L hydrochloric acid TS and in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Aciclovir in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum or the spectrum of Aciclovir Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aciclovir as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum or the spectrum of Aciclovir Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Aciclovir in 20 mL of dilute sodium hydroxide TS: the solution is clear and is not more colored than the following control solution.

Control solution: To 2.5 mL of Matching Fluid for Color F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals *<1.07>*—Proceed with 1.0 g of Aciclovir 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).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 25 mg of guanine, dissolve in 50 mL of dilute sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 2 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 standard solution as directed under Liquid Chro-

matography <*2.01*> according to the following conditions. Determine the peak areas of guanine, A_T and A_S, and calculate the amount of guanine by the following equation: it is not more than 0.7%. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of each related substance other than aciclovir and guanine by the area percentage method: it is not more than 0.2%. Furthermore, the sum of the amount of guanine calculated above and the amounts of related substances determined by the area percentage method is not more than 1.5%.

$$\text{Amount (\%) of guanine} = (W_S/W_T) \times (A_T/A_S) \times (2/5)$$

W_S: Amount (mg) of guanine
W_T: Amount (mg) of Aciclovir

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 8 times as long as the retention time of aciclovir beginning after the solvent peak.

System suitability—

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

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 aciclovir obtained from 10 μL of this solution is equivalent to 7 to 13% of that from the solution for system suitability test.

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 guanine is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 6.0% (50 mg, potentiometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Aciclovir and Aciclovir Reference Standard (separately determine the water <*2.48*> in the same manner as Aciclovir), dissolve each in 1 mL of dilute sodium hydroxide TS, add the mobile phase to make exactly 20 mL each, and use these solutions as the sample solution and standard solutions, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of aciclovir in each solution.

$$\text{Amount (mg) of } C_8H_{11}N_5O_3 = W_S \times (A_T/A_S)$$

*W*s: Amount (mg) of Aciclovir Reference Standard, calculated on the anhydrous basis

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 (3 μm in particle diameter).

Column temperature: A constant temperature of about 20ºC.

Mobile phase: Dissolve 1.0 g of sodium 1-decanesulfonate and 6.0 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To this solution add 40 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of aciclovir is about 3 minutes.

System suitability—

System performance: Dissolve 0.1 g of Aciclovir in 5 mL of dilute sodium hydroxide TS, add 2 mL of a solution of guanine in dilute sodium hydroxide TS (1 in 4000), and add the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, aciclovir and guanine are eluted in this order with the resolution between these peaks being not less than 17.

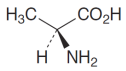
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aciclovir is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

L-Alanine

L - アラニン



$C_3H_7NO_2$: 89.09
(2*S*)-2-Aminopropanoic acid [*56-41-7*]

L-Alanine, when dried, contains not less than 98.5% and not more than 101.0% of $C_3H_7NO_2$.

Description L-Alanine occurs as white crystals or crystalline powder. It has a slightly sweet taste.

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

It dissolves in 6 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Alanine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +13.5 - +15.5º (after drying, 2.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of L-Alanine in 20 mL of water: the pH of the solution is between 5.7 and 6.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Alanine in 10 mL of water: the solution is clear and colorless.

(2) Chloride *<1.03>*—Perform the test with 0.5 g of L-Alanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate *<1.14>*—Perform the test with 0.6 g of L-Alanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium *<1.02>*—Perform the test with 0.25 g of L-Alanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals *<1.07>*—Proceed with 1.0 g of L-Alanine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron *<1.10>*—Prepare the test solution with 1.0 g of L-Alanine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Alanine, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately measure 2.5 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-valine, L-cystine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate, and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Based on the peak heights obtained from the sample solution and standard solution, determine the mass of the amino acids other than alanine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acid other than alanine is not more than 0.1%.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 570 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57ºC.

Chemical reaction bath temperature: A constant temperature of about 130ºC.

Reaction time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and add 0.1 mL of capric acid to each mobile phase.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	-
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	-
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	-
Sodium hydroxide	-	-	-	-	8.00 g
Ethanol (99.5)	260 mL	20 mL	4 mL	-	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	-	-
Benzyl alcohol	-	-	-	5 mL	-
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount
Total volume	2000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing mobile phases: When the procedure is run with 20 μL of the standard solution under the above operating conditions, switchover to mobile phase A, mobile phase B, mobile phase C, mobile phase D and mobile phase E, in sequence so that aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagents: Dissolve 407 g of lithium acetate dehydrate in water, add 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, and water to make 2000 mL, introduce nitrogen for 10 minutes, and use this solution as solution (I). Separately, add 77 g of ninhydrin to 1957 mL of 1-methoxy-2-propanol, introduce nitrogen for 5 minutes, add 0.134 g of sodium borohydride, and introduce nitrogen for 30 minutes. To 12 volumes of this solution add 13 volumes of solution (I). Prepare before use.

Flow rate of mobile phase: 0.32 mL per minute.

Flow rate of reaction reagent: 0.30 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is 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 deviations of the peak height and retention time of each amino acid obtained from the standard solution are not more than 5.0% and not more than 1.0%, respectively.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105ºC, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 90 mg of L-Alanine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.909 mg of $C_3H_7NO_2$

Containers and storage Containers—Tight containers.

Add the following:

Allopurinol Tablets

アロプリノール錠

Allopurinol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of allopurinol ($C_5H_4N_4O$: 136.11).

Method of preparation Prepare as directed under Tablets, with Allopurinol.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 248 nm and 252 nm.

(2) To a quantity of powdered Allopurinol Tablets, equivalent to 0.1 g of Allopurinol according to the labeled amount, add 5 mL of a solution of diethylamine (1 in 10), shake well, add 5 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.1 g of allopurinol in 5 mL of a solution of diethylamine (1 in 10), add 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2.5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, ammonia solution (28) and 2-methoxyethanol (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the sample solution and standard solution show the same R_f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Allopurinol Tablets add V/10 mL of 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of allopurinol ($C_5H_4N_4O$), and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L of hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of allopurinol for assay, previously dried at 105ºC for 4 hours, dissolve in 10 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of allopurinol ($C_5H_4N_4O$)
$= W_S \times (A_T/A_S) \times (V/100)$

W_S: Amount (mg) of allopurinol for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Allopurinol Tablets is not less than 80%.

Start the test with 1 tablet of Allopurinol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of allopurinol ($C_5H_4N_4O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 11 mg of allopurinol for assay, previously dried at 105ºC for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of allopurinol ($C_5H_4N_4O$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

W_S: Amount (mg) of allopurinol for assay
C: Labeled amount (mg) of allopurinol ($C_5H_4N_4O$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Allopurinol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of allopurinol ($C_5H_4N_4O$), add

20 mL of 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of allopurinol for assay, previously dried at 105ºC for 4 hours, dissolve in 20 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Amount (mg) of allopurinol ($C_5H_4N_4O$) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of allopurinol for assay

Containers and storage Containers—Well-closed containers.

Dried Aluminum Hydroxide Gel Fine Granules

乾燥水酸化アルミニウムゲル細粒

Change the Method of preparation to read:

Method of preparation Prepare to finely granulated form as directed under Powders, with Dried Aluminum Hydroxide Gel.

Add the following next to the Identification:

Particle size *<6.03>* It meets the requirement.

Aminophylline Injection

アミノフィリン注射液

Change the Assay (1) to read:

Assay (1) Theophylline—Pipet a volume of Aminophylline Injection, equivalent to about 39.4 mg of theophylline ($C_7H_8N_4O_2$) (about 50 mg of Aminophylline Hydrate), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of theophylline for assay, previously dried at 105ºC for 4 hours, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of theophylline in each solution.

Amount (mg) of theophylline ($C_7H_8N_4O_2$)

$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of theophylline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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 40ºC.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

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

System suitability—

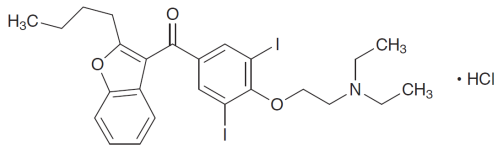
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.

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 peak area of theophylline is not more than 1.0%.

Add the following:

Amiodarone Hydrochloride

アミオダロン塩酸塩



$C_{25}H_{29}I_2NO_3$·HCl: 681.77

(2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl}methanone monohydrochloride [*19774-82-4*]

Amiodarone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{25}H_{29}I_2NO_3$·HCl.

Description Amiodarone Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is very soluble in water at 80ºC, freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Melting point: about 161ºC (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Amiodarone Hydrochloride in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Amiodarone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Amiodarone Hydrochloride add 10 mL of water, dissolve by warming at 80ºC, and cool: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> To 1.0 g of Amiodarone Hydrochloride add 20 mL of freshly boiled and cooled water, dissolve by warming at 80ºC, and cool: the pH of this solution is between 3.2 and 3.8.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Amiodarone Hydrochloride in 10 mL of methanol: the solution is clear, and is not more colored than the following control solutions (1) and (2).

Control solution (1): To a mixture of 1.0 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 2.4 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.4 mL of Copper (II) Sulfate Colorimetric Stock Solution, add diluted hydrochloric acid (1 in 40) to make 10.0 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 20 mL.

Control solution (2): To 3.0 mL of a mixture of 0.2 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 9.6 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.2 mL of Copper (II) Sulfate Colorimetric Stock Solution, add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Iodine—To 1.50 g of Amiodarone Hydrochloride add 40 mL of water, dissolve by warming at 80ºC, cool, add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 15 mL of this solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS and exactly 1 mL of a solution of potassium iodate (107 in 10000), add water to make exactly 20 mL, and use this solution as the sample solution. Separately, pipet 15 mL of the sample stock solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, exactly 1 mL of a solution of potassium iodide (441 in 5000000) and exactly 1 mL of a solution of potassium iodate (107 in 10000), add water to make exactly 20 mL, and use this solution as the standard solution. Separately, pipet 15 mL of the sample stock solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 20 mL, and use this solution as the control solution. Allow the sample solution, standard solution and control solution to stand in a dark place for 4 hours. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control solution as the blank: the absorbance of the sample solution at 420 nm is not larger than 1/2 times the absorbance of the standard solution.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Amiodarone 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).

(4) Related substance 1—Dissolve 0.5 g of Amiodarone Hydrochloride in 5 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-chloroethyl diethylamine hydrochloride in 50 mL of dichloromethane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and formic acid (17:2:1) to a distance of about 15 cm, and air-dry the plate. Splay evenly bismuth subnitrate TS and then hydrogen peroxide TS: the spot obtained from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

(5) Related substance 2—Dissolve 0.125 g of Amiodarone Hydrochloride in 25 mL of a mixture of water and acetonitrile for liquid chromatography (1:1), and use this solution as the sample solution. Pipet 2 mL of this solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (1:1) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of any peak other than amiodarone obtained from the sample solution is not larger than the peak area of amiodarone from the standard solution, and the total area of the peaks other than the peak of amiodarone from the sample solution is not larger than 2.5 times the peak area of amiodarone 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 30ºC.

Mobile phase: To 800 mL of water add 3.0 mL of acetic acid (100), adjust the pH to 4.95 with ammonia solution (28), and add water to make 1000 mL. To 300 mL of this solution add 400 mL of acetonitrile for liquid chromatography and 300 mL of methanol for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of amiodarone is about 24 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 25 mL. Confirm that the peak area of amiodarone obtained from 10 μL of this solution is equivalent to 14 to 26% of that of amiodarone from 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 factor of the peak of amiodarone 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 amiodarone is not more than 1.0%.

(6) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, reduced pressure not exceeding 0.3 kPa, 50ºC, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Amiodarone Hydrochloride, previously dried, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (3:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.18 mg of $C_{25}H_{29}I_2NO_3$·HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Amiodarone Hydrochloride Tablets

アミオダロン塩酸塩錠

Amiodarone Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl: 681.77).

Method of preparation Prepare as directed under Tablets, with Amiodarone Hydrochloride.

Identification To 1 mL of the sample stock solution obtained in the Assay add the mobile phase to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 239 nm and 243 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amiodarone Hydrochloride Tablets add 160 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, add the mobile phase to make exactly 200 mL, and centrifuge. Pipet *V* mL of the supernatant liquid, equivalent to about 1 mg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl), add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of amiodarone hydrochloride for assay, previously dried at 50ºC for 4 hours under reduced pressure not exceeding 0.3 kPa, 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amiodarone in each solution.

Amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl)
$= W_S \times (A_T/A_S) \times (8/V)$

W_S: Amount (mg) of amiodarone for assay

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

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 factor of the peak of amiodarone 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 amiodarone is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 30 minutes of Amiodarone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Amiodarone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add methanol to make exactly *V*′ mL so that each mL contains about 11 μg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of amiodarone hydrochloride for assay, previously dried at 50ºC for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the dissolution medium, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared by adding methanol to 2 mL of the dissolution medium to make 20 mL, as the blank.

Dissolution rate (%) with respect to the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of amiodarone for assay
C: Labeled amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Amiodarone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of amiodarone

hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl), add 80 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample stock solution. Pipet 2 mL of this solution, add 2 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately 25 mg of amiodarone hydrochloride for assay, previously dried at 50ºC for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amiodarone to that of the internal standard.

Amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$·HCl)
$= W_S \times (Q_T/Q_S) \times 2$

W_S: Amount (mg) of amiodarone hydrochloride for assay

Internal standard solution—A solution of chlorhexidine hydrochloride in the mobile phase (1 in 2500)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 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 50ºC.

Mobile phase: A mixture of acetonitrile for liquid chromatography, a solution of sodium laurylsulfate (1 in 50) and phosphoric acid (750:250:1).

Flow rate: Adjust the flow rate so that the retention time of amiodarone 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 amiodarone 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 ratio of the peak area of amiodarone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Amlodipine Besilate Tablets

アムロジピンベシル酸塩錠

Amlodipine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amlodipine besilate ($C_{20}H_{25}ClN_2O_5·C_6H_6O_3S$: 567.05).

Method of preparation Prepare as directed under Tablets, with Amlodipine Besilate.

Identification To a quantity of powdered Amlodipine Besilate Tablets, equivalent to 2.5 mg of Amlodipine Besilate according to the labeled amount, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake vigorously, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 235 nm and 239 nm, and between 358 nm and 362 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amlodipine Besilate Tablets add 10 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly V mL so that each mL contains about 69 μg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5·C_6H_6O_3S$), and shake for 60 minutes. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of amlodipine besilate ($C_{20}H_{25}ClN_2O_5·C_6H_6O_3S$)
$= W_S \times (Q_T/Q_S) \times (V/500)$

W_S: Amount (mg) of Amlodipine Besilate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20000)

Dissolution Being specified separately.

Assay To 20 Amlodipine Besilate Tablets add 100 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly 1000 mL, and shake for 60 minutes. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 0.7 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5·C_6H_6O_3S$), add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besilate Reference Standard (separately, determine the water <*2.48*> in the same manner as Amlodipine Besilate), and dissolve in the mobile phase to make exactly 250 mL. Pipet 5 mL of this solution, add

exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlodipine to that of the internal standard.

Amount (mg) of amlodipine besilate ($C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$)
$= W_S \times (Q_T/Q_S) \times (1/50)$

W_S: Amount (mg) of Amlodipine Besilate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 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 25ºC.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate (41 in 10000) (13:7)

Flow rate: Adjust the flow rate so that the retention time of amlodipine 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, amlodipine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amlodipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Amoxicillin Capsules

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Amoxicillin Capsules contain not less than 92.0% and not more than 105.0% of the labeled potency of Amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of preparation Prepare as directed under Capsules, with Amoxicillin Hydrate.

Identification Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 8 mg (potency) of Amoxicillin Hydrate according to the labeled amount, add 2 mL of 0.01 mol/L hydrochloric acid TS, shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve an amount equivalent to 8 mg (potency) of Amoxicillin Reference Standard in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, water and formic acid (50:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 20) on the plate, and heat the plate at 110ºC for 15 minutes: the principal spot obtained from the sample solution and the standard solution show a red-purple color and the same R_f value.

Purity Related substances— Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 0.1 g (potency) of Amoxicillin Hydrate according to the labeled amount, add 30 mL of a solution of boric acid (1 in 200), shake for 15 minutes, and add a solution of boric acid (1 in 200) to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of this solution, add a solution of boric acid (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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Amoxicillin Hydrate.

System suitability—

Test for required detectability and system repeatability: Proceed as directed in the system suitability in the Purity (3) under Amoxicillin Hydrate.

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 factor of the peak of amoxicillin is not less than 2500 and not more than 1.5, respectively.

Water *<2.48>* Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Uniformity of dosage units *<6.02>* It meets the requirement of the Mass variation test.

Dissolution *<6.10>* When the test is performed at 100 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Amoxicillin Capsules is not less than 75%.

Start the test with 1 capsule of Amoxicillin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the

filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg (potency) of Amoxicillin Hydrate according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount equivalent to about 28 mg (potency) of Amoxicillin Reference Standard, dissolve in water to make exactly 100 mL. Pipet 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 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in each solution.

Dissolution rate (%) with respect to the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$

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

C: Labeled amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 2.0, respectively.

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 amoxicillin is not more than 1.5%.

Assay Weigh accurately the mass of not less than 10 Amoxicillin Capsules, take out the contents, and weigh accurately the mass of the emptied shells. Weigh accurately an amount equivalent to about 0.1 g (potency) of Amoxicillin Hydrate according to the labeled amount, add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount equivalent to about 20 mg (potency) of Amoxicillin Reference Standard, dissolve in water 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in each solution.

Amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$)
$= W_S \times (A_T/A_S) \times 5$

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

Operating conditions—

Column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

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).

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 factor of the peak of amoxicillin are not less than 2500 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 peak areas of amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amoxicillin Hydrate

アモキシシリン水和物

Change the Purity (3) to read:

Purity (3) Related substances—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of boric acid (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger 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 25ºC.

Mobile phase: Dissolve 1.36 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 detectability: To exactly 1 mL of the standard solution add a solution of boric acid (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin ob-

tained from 10 μL of this solution is equivalent to 7 to 13% of that of amoxicillin from the standard solution.

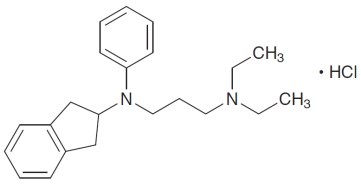
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

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 amoxicillin is not more than 1.0%.

Add the following:

Aprindine Hydrochloride

アプリンジン塩酸塩



$C_{22}H_{30}N_2 \cdot HCl$: 358.95
N-(2,3-Dihydro-1*H*-inden-2-yl)-*N'*,*N'*-diethyl-*N*-phenylpropane-1,3-diamine monohydrochloride
[*33237-74-0*]

Aprindine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{22}H_{30}N_2 \cdot HCl$.

Description Aprindine Hydrochloride occurs as a white to pale yellowish white crystalline powder. It has a bitter taste, numbing the tongue.

It is very soluble in water, methanol and in acetic acid (100), and freely soluble in ethanol (99.5).

It gradually turns brown on exposure to light.

Identification **(1)** Dissolve 10 mg of Aprindine Hydrochloride in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Aprindine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Aprindine Hydrochloride (1 in 50) add 1 mL of dilute nitric acid: this solution responds to the Qualitative Tests *<1.09>* for chloride.

pH *<2.54>* Dissolve 1.0 g of Aprindine Hydrochloride in 50 mL of water: the pH of the solution is between 6.4 and 7.0.

Melting point *<2.60>* 127-131ºC

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aprindine Hydrochloride in 10 mL of methanol: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry *<2.24>* is not more than 0.10.

(2) Heavy metals *<1.07>*—Proceed with 1.0 g of Aprindine Hydrochloride 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).

(3) Related substances—Dissolve 25 mg of Aprindine Hydrochloride in 10 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 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than aprindine obtained from the sample solution is not larger than 1/10 times the peak area of aprindine 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 40ºC.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.

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

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aprindine obtained from 10 μL of this solution is equivalent to 7 to 13% of that of aprindine from 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 factor of the peak of aprindine 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 aprindine is not more than 1.5%.

(4) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, in vacuum, 60ºC, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Aprindine Hydrochloride, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.90 mg of $C_{22}H_{30}N_2$·HCl

Containers and storage Containers—Well-closed containers
Storage—Light-resistant.

Add the following:

Aprindine Hydrochloride Capsules

アプリンジン塩酸塩カプセル

Aprindine Hydrochloride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl: 358.95).

Method of preparation Prepare as directed under Capsules, with Aprindine Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, it exhibits maxima between 264 nm and 268 nm, and between 271 nm and 275 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Aprindine Hydrochloride Capsules, add 30 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125), shake vigorously for 20 minutes, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly V mL so that each mL contains about 0.2 mg of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl), and filter. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl)
$= W_S \times (A_T/A_S) \times (V/250)$

W_S: Amount (mg) of aprindine hydrochloride for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Aprindine Hydrochloride Capsules is not less than 80%.

Start the test with 1 capsule of Aprindine Hydrochloride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60ºC for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of aprindine in each solution.

Dissolution rate (%) with respect to the labeled amount of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of aprindine hydrochloride for assay

C: Labeled amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl) in 1 capsule

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 40ºC.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.

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

System suitability—

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 factor of the peak of aprindine are not less than 3000 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 aprindine is not more than 1.5%.

Assay Take out the contents of not less than 20 Aprindine Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aprindine hydrochloride ($C_{22}H_{30}N_2$·HCl), add 60 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125), shake vigorously for 20 minutes, and add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 100 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and filter, Discard the first 5 mL of

the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60ºC for 4 hours, and dissolve in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 265 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2 \cdot HCl$)
$= W_S \times (A_T/A_S) \times 2$

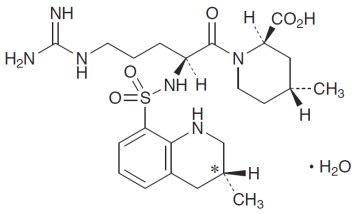
W_S: Amount (mg) of aprindine hydrochloride for assay

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Add the following:

Argatroban Hydrate

アルガトロバン水和物



and epimer at C*

$C_{23}H_{36}N_6O_5S \cdot H_2O$: 526.65
(2*R*,4*R*)-4-Methyl-1-((2*S*)-2-{[(3*RS*)-3-methyl-1,2,3,4-tetrahydroquinolin-8-yl]sulfonyl}amino-5-guanidinopentanoyl)piperidine-2-carboxylic acid monohydrate [*141396-28-3*]

Argatroban Hydrate contains not less than 98.5% and not more than 101.0% of argatroban ($C_{23}H_{36}N_6O_5S$: 508.63), calculated on the anhydrous basis.

Description Argatroban Hydrate occurs as white crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

It is gradually decomposed on exposure to light.

Identification (1) Determine the absorption spectrum of a solution of Argatroban Hydrate in ethanol (99.5) (1 in 20000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Argatroban Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +175 - +185º (0.2 g calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Argatroban Hydrate 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 <*1.11*>—Incinerate 2.0 g of Argatroban Hydrate according to Method 4. After cooling, 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. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 1 ppm).

(3) Related substance 1—Dissolve 50 mg of Argatroban Hydrate in 40 mL of methanol, add water 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 Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than argatroban is not more than 0.1%.

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 45ºC.

Mobile phase A: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 500 mL of this solution add 500 mL of methanol.

Mobile phase B: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 200 mL of this solution add 800 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 (vol%)	Mobile phase B (vol%)
0-5	100	0
5-35	100→5	0→95

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 1.5 times as long as the retention time of argatroban beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase A 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 A to

make exactly 10 mL. Confirm that the peak area of argatroban obtained from 10 μL of this solution is equivalent to 7 to 13% of that of argatroban from the solution for system suitability test.

System performance: Dissolve 5 mg of Argatroban Hydrate and 5 μL of methyl benzoate in 40 mL of methanol, and add water to make 100 mL. To 5 mL of this solution add 40 mL of methanol and water to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl benzoate and argatroban 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 argatroban is not more than 2.0%.

(4) Related substance 2— Dissolve 0.10 g of Argatroban Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of this solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate and water (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of spots other than the principal spot obtained from the sample solution is not more than 2, and they are not more intense than the spot from the standard solution.

(5) Residual solvent—Being specified separately.

Water *<2.48>* 2.5 - 4.5% (0.2 g, volumetric titration, direct titration).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Isomer ratio Dissolve 50 mg of Argatroban Hydrate in 50 mL of methanol, add water 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 Liquid Chromatography *<2.01>* according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention times of about 40 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 0.30 and 0.40.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 40ºC.

Mobile phase: To 500 mL of water add 500 mL of methanol, 13 mL of diluted 40% tetrabutylammonium hydroxide TS (1 in 4) and 0.68 mL of phosphoric acid, and adjust the pH to 6.8 with ammonia TS and diluted ammonia solution (28) (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of the peak having the shorter retention time of the two peaks of argatroban is about 40 minutes.

System suitability—

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 is not less than 1.2.

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 total area of the two separate peaks of argatroban is not more than 2.0%.

Assay Weigh accurately about 0.5 g of Argatroban Hydrate, dissolve in 20 mL of acetic acid for nonaqueous titration, add 40 mL of acetone for nonaqueous titration, and titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.86 mg of $C_{23}H_{36}N_6O_5S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Azathioprine Tablets

アザチオプリン錠

Add the following next to the Identification:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Azathioprine Tablets add 1 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry per 5 mg of Azathioprine ($C_9H_7N_7O_2S$), shake well, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.2 mg of azathioprine ($C_9H_7N_7O_2S$), and filter. Discard the first 20 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of azathioprine ($C_9H_7N_7O_2S$)
$= W_S \times (A_T/A_S) \times (V/500)$

W_S: Amount (mg) of Azathioprine Reference Standard

Add the following:

Azelastine Hydrochloride Granules

アゼラスチン塩酸塩顆粒

Azelastine Hydrochloride Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl: 418.36).

Method of preparation Prepare as directed under Granules, with Azelastine Hydrochloride.

Identification To a quantity of Azelastine Hydrochloride Granules, equivalent to 2 mg of Azelastine Hydrochloride according to the labeled amount, add 30 mL of 0.1 mol/L hydrochloric acid TS, and treat with ultrasonic waves for 30 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 283 nm and 287 nm.

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 45 minutes of Azelastine Hydrochloride Granules is not less than 80%.

Start the test with accurately weighed amount of Azelastine Hydrochloride Granules, equivalent to about 1 mg of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl) according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105ºC for 2 hours, and dissolve in the dissolution medium to make exactly 250 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of azelastine in each solution.

Dissolution rate (%) with respect to the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times (9/5)$

W_S: Amount (mg) of azelastine hydrochloride for assay
W_T: Amount (g) of Azelastine Hydrochloride Granules
C: Labeled amount (mg) of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl) in 1 g

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine are not less than 2000 and not more than 1.5, respectively.

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 azelastine is not more than 2.0%.

Particle size *<6.03>* It meets the requirement.

Assay Weigh accurately an amount of Azelastine Hydrochloride Granules, equivalent to about 2 mg of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl), add 50 mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves for 20 minutes, add 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, and add ethanol (99.5) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105ºC for 2 hours, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 40 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, add ethanol (99.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azelastine to that of the internal standard.

Amount (mg) of azelastine hydrochloride ($C_{22}H_{24}ClN_3O$·HCl)
$= W_S \times (Q_T/Q_S) \times (1/25)$

W_S: Amount (mg) of azelastine hydrochloride for assay

Internal standard solution—Dissolve 0.2 g of 2-ethylhexyl parahydroxybenzoate in ethanol (99.5) to make 100 mL.

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 40ºC.

Mobile phase: A mixture of acetonitrile and a solution of sodium lauryl sulfate in diluted acetic acid (100) (1 in 250) (1 in 500) (11:9).

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

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, azelastine 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 ratio of the peak area of azelastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone Tablets

ベタメタゾン錠

Change the Uniformity of dosage units to read:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Betamethasone Tablets add *V* mL of water so that each mL contains about 50 μg of betamethasone ($C_{22}H_{29}FO_5$). Add exactly 2 *V* mL of the internal standard solution, shake vigorously for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone Reference Standard, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, add 5 mL of water, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>*, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = W_S \times (Q_T/Q_S) \times (V/400)$$

W_S: Amount (mg) of Betamethasone Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 40000)

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

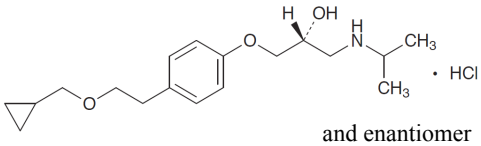
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

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

Add the following:

Betaxolol Hydrochloride

ベタキソロール塩酸塩



and enantiomer

$C_{18}H_{29}NO_3 \cdot HCl$: 343.89

(2*RS*)-1-{4-[2-(Cyclopropylmethoxy)ethyl]phenoxy}-3-[(1-methylethyl)amino]propan-2-ol monohydrochloride [*63659-19-8*]

Betaxolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{18}H_{29}NO_3 \cdot HCl$.

Description Betaxolol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol, ethanol (99.5) or acetic acid (100).

Dissolve 1.0 g of Betaxolol Hydrochloride in 50 mL of water: the pH of the solution is between 4.5 and 6.5.

A solution of Betaxolol Hydrochloride (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Betaxolol Hydrochloride in ethanol (99.5) (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Betaxolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Betaxolol Hydrochloride (1 in 10) responds to the Qualitative Tests *<1.09>* (2) for chloride.

Melting point *<2.60>* 114-117ºC

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Betaxolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Betaxolol Hydrochloride 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 *<1.11>*—Prepare the test solution with 2.0 g of Betaxolol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substance I—Dissolve 0.10 g of Betaxolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of this solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add metha-

nol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (10:3:3) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 1 hour: 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.

(5) Related substance II—Dissolve 0.10 g of Betaxolol 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 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than betaxolol obtained from the sample solution is not larger than the peak area of betaxolol from the standard solution, and the total area of the peaks other than the peak of betaxolol obtained from the sample solution is not larger than 2 times the peak area of betaxolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 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 25ºC.

Mobile phase: A mixture of diluted 0.05 mol/L potassium dihydrogen phosphate TS (1 in 2) with the pH adjusted to 3.0 with 1 mol/L hydrochloric acid TS, acetonitrile and methanol (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of betaxolol is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of betaxolol beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of betaxolol obtained from 10 μL of this solution is equivalent to 14 to 26% of that of bataxolol from the standard solution.

System performance: Dissolve 50 mg of Betaxolol Hydrochloride and 5 mg of 2-naphthol in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, betaxolol and 2-naphthol 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 peak area of betaxolol is not more than 2.0%.

(6) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Betaxolol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

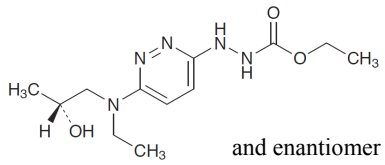
Each mL of 0.1 mol/L perchloric acid VS
= 34.39 mg of $C_{18}H_{29}NO_3 \cdot HCl$

Containers and storage Containers—Tight containers.

Add the following:

Cadralazine

カドララジン



and enantiomer

$C_{12}H_{21}N_5O_3$: 283.33

Ethyl 3-(6-{ethyl[(2*RS*)-2-hydroxypropyl]amino}pyridazin-3-yl)carbazate [*64241-34-5*]

Cadralazine, when dried, contains not less than 98.5% and not more than 101.0% of $C_{12}H_{21}N_5O_3$.

Description Cadralazine occurs as a pale yellow to light yellow crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in 0.05 mol/L sulfuric acid TS.

A solution of Cadralazine in methanol (1 in 40) shows no optical rotation.

Melting point: about 165ºC (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Cadralazine in 0.05 mol/L sulfuric acid TS (1 in 125000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Cadralazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <*1.03*>—Dissolve 0.40 g of Cadralazine

in 15 mL of methanol, 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 with 0.40 mL of 0.01 mol/L hydrochloric acid VS by adding 15 mL of methanol, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cadralazine 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) Related substances—Dissolve 50 mg of Cadralazine in 20 mL of 0.05 mol/L sulfuric acid TS, add water to 100 mL, and use this solution as the sample solution. Pipet 1 mL of this 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.1 with respect to cadralazine, obtained from the sample solution is not larger than the peak area of cadralazine from the standard solution, and the area of the peak other than cadralazine and other than the peak mentioned above is not larger than 2/5 times the peak area of cadralazine from the standard solution. Furthermore, the total area of the peaks other than cadralazine obtained from the sample solution is not larger than 2 times the peak area of cadralazine from the standard solution. For this calculation, use the areas of the peaks, having the relative retention time of about 0.49 and about 2.1 with respect to cadralazine, after multiplying by their relative response factors, 0.65 and 1.25, respectively.

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 40ºC.

Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 800 mL of water, adjust the pH to 5.8 with dilute acetic acid, and add water to make 1000 mL. To 860 mL add 140 mL of acetonitrile.

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

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of cadralazine obtained from 10 μL of this solution is equivalent to 15 to 25% of that of cadralazine from 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 factor of the peak of cadralazine are not less than 4000 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 cadralazine is not more than 4.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105ºC, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Cadralazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.33 mg of $C_{12}H_{21}N_5O_3$

Containers and storage Containers—Well-closed containers.

Add the following:

Cadralazine Tablets

カドララジン錠

Cadralazine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cadralazine ($C_{12}H_{21}N_5O_3$: 283.33).

Method of preparation Prepare as directed under Tablets, with Cadralazine.

Identification To a quantity of powdered Cadralazine Tablets, equivalent to 20 mg of Cadralazine according to the labeled amount, add 50 mL of 0.05 mol/L sulfuric acid TS, shake well, and centrifuge. To 1 mL of the supernatant liquid add 0.05 mol/L sulfuric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 247 nm and 251 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cadralazine Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, and add 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 6 μg of cadralazine ($C_{12}H_{21}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of cadralazine for assay, previously dried at 105ºC for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample so-

lution and standard solution at 249 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of cadralazine } (C_{12}H_{21}N_5O_3) = W_S \times (A_T/A_S) \times (V/200)$$

W_S: Amount (mg) of cadralazine for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cadralazine Tablets is not less than 80%.

Start the test with 1 tablet of Cadralazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of cadralazine ($C_{12}H_{21}N_5O_3$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of cadralazine for assay, previously dried at 105ºC for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cadralazine } (C_{12}H_{21}N_5O_3) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S: Amount (mg) of cadralazine for assay
C: Labeled amount (mg) of cadralazine ($C_{12}H_{21}N_5O_3$) in 1 tablet

Assay To 10 Cadralazine Tablets add 70 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, add 0.05 mol/L sulfuric acid to make exactly 200 mL. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 2.5 mg of cadralazine ($C_{12}H_{21}N_5O_3$), add exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of cadralazine for assay, previously dried at 105ºC for 3 hours, and dissolve in 0.05 mol/L of sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cadralazine to that of the internal standard.

$$\text{Amount (mg) of cadralazine } (C_{12}H_{21}N_5O_3) = W_S \times (Q_T/Q_S) \times (1/10)$$

W_S: Amount (mg) of cadralazine for assay

Internal standard solution—A solution of *p*-toluenesulfonamide in acetonitrile (1 in 50)

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 40ºC.

Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 800 mL of water, adjust the pH to 5.8 with dilute acetic acid, and add water to make 1000 mL. To 860 mL of this solution add 140 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 5 μL of this solution under the above operating conditions, cadralazine 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 ratio of the peak area of cadralazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Calcitonin (Salmon)

カルシトニン（サケ）

CSNLSTCVLG KLSQELHKLQ TYPRTNTGSG TP-NH_2

$C_{145}H_{240}N_{44}O_{48}S_2$: 3431.85
[*47931-85-1*]

Calcitonin (Salmon) is a synthetic polypeptide consisting of 32 amino acid residues. It is a hormone with a blood calcium lowering effect.

It contains not less than 4000 Units of calcitonin (salmon) per 1 mg of peptide.

Description Calcitonin (Salmon) occurs as a white powder.

It is freely soluble in water.

It dissolves in dilute acetic acid.

Dissolve 20 mg of Calcitonin (Salmon) in 2 mL of water: the pH of the solution is between 5.0 and 7.0.

It is hygroscopic.

Identification Dissolve 1 mg of Calcitonin (Salmon) in 1 mL of dilute acetic acid. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <*2.24*> $E_{1cm}^{1\%}$ (275 nm): 3.3 - 4.0 (1 mg, dilute acetic acid, 1 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: –24 - –32º (25 mg, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm).

Constituent amino acids Weigh accurately about 1 mg of Calcitonin (Salmon), put in a test tube for hydrolysis, dissolve in 0.5 mL of diluted hydrochloric acid (1 in 2), freeze in a dry ice-acetone bath, seal the tube under reduced pressure, and heat at 110 ± 2ºC for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in exactly 5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid TS 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: 13 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios with respect to leucine (=5) are 1.9 – 2.3 for lysine, 0.8 – 1.1 for histidine, 0.9 – 1.1 for arginine, 1.9 – 2.1 for aspartic acid, 4.5 – 4.9 for threonine, 3.2 – 3.8 for serine, 2.8 – 3.1 for glutamic acid, 1.9 – 2.4 for proline, 2.7 – 3.3 for glycine, 1.5 – 2.5 for 1/2 cystine, 0.9 – 1.0 for valine, and 0.8 – 1.0 for tyrosine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 6 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57ºC.

Chemical reaction bath temperature: A constant temperature of about 130ºC.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	–
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	–
Sodium hydroxide	–	–	–	–	8.00 g
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	–
Ethanol (99.5)	130.0 mL	20.0 mL	4.0 mL	–	100.0 mL
Benzyl alcohol	–	–	–	5.0 mL	–
Thiodiglycol	5.0 mL	5.0 mL	5.0 mL	–	–
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)	Mobile phase E (vol%)
0 – 1.5	100	0	0	0	0
1.5 – 4	0	100	0	0	0
4 – 12	0	0	100	0	0
12 – 26	0	0	0	100	0
26 – 30	0	0	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for 10 minutes while passing nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for 30 minutes while passing nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: About 0.4 mL per minute.

Flow rate of reaction reagent: About 0.35 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.2, 1.0 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

Peptide content Calculate the peptide content in Calcitonin (Salmon) by the following equation using amino acid analysis values (μmol/mL) obtained in the Constituent amino acids: it is not less than 80.0%.

$$\text{Peptide content (\%)} = 3431.85 \times (5/W) \times (A/11) \times 100$$

A: Total (μmol/mL) of the amino acid analysis values of valine, leucine, glycine and proline

W: Amount (μg) of sample

11: Total of the theoretical residue numbers of valine, leucine, glycine and proline per one mole of calcitonin (salmon)

Purity (1) Acetic acid—Weigh accurately about 10 mg of Calcitonin (Salmon), dissolve in water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of acetic acid in each solution, and calculate the amount of acetic acid by the following equation: the amount of acetic acid is not more than 7.0%.

$$\text{Amount (\%) of acetic acid } (CH_3COOH) = (W_S/W_T) \times (A_T/A_S) \times (1/10)$$

W_S: Amount (mg) of acetic acid (100)

W_T: Amount (mg) of Calcitonin (Salmon)

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 40ºC.

Mobile phase A: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Mobile phase B: 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 (vol%)	Mobile phase B (vol%)
0 – 5	95	5
5 – 10	95→50	5→50
10 – 20	50	50
20 – 22	50→95	50→5
22 – 30	95	5

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ace-

tic acid is not more than 2.0%.

(2) Related substances—Dissolve 2 mg of Calcitonin (Salmon) in 2 mL of dilute acetic acid, and use this solution as the sample solution. Perform the test with 20 μL of this solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than calcitonin (salmon) is not more than 3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 25ºC.

Mobile phase: A mixture of 1% trimethylamine-phosphate buffer solution, pH 3.0, and acetonitrile (27:13).

Flow rate: Adjust the flow rate so that the retention time of calcitonin (salmon) is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of calcitonin (salmon) beginning 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 calcitonin (salmon) obtained from 20 μL of this solution is equivalent to 5 to 15% of that of calcitonin (salmon) from the solution for system suitability test.

System performance: Dissolve 5 mg of methyl parahydroxybenzoate and 7 mg of ethyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 20 μL of this solution under the above operating conditions methyl parahydroxybenzoate and ethyl parahydroxybenzoate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of calcitonin (salmon) is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 10.0% (5 mg, coulometric titration).

Assay (i) Test animals: Select healthy albino rats weighing between 55 and 180 g, fasted for 24 hours before the test but allowed to drink water ad libitum..

(ii) Standard solutions: Dissolve a quantity of Calcitonin (Salmon) Reference Standard in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L containing exactly 0.050 and 0.025 Units per mL, respectively.

(iii) Sample solutions: According to the labeled units, weigh accurately a suitable amount of Calcitonin (Salmon), and dissolve in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose sample solution T_H and the low-dose sample solution T_L having Units equal to the standard solutions in equal volumes, respectively.

(iv) Dose for injection: Inject 0.3 mL per animal.

(v) Procedure: Divide the test animals at random into 4 groups, A, B, C and D, with not less than 8 animals and equal numbers in each group. Inject S_H, S_L, T_H and T_L into the tail vein or subcutaneously into the neck of each animal of the respective groups. At 1 hour after the injection, collect blood from the abdominal aorta in a way that minimizes the suffering of the animals, allow the blood samples to stand at room temperature for about 30 minutes, and centrifuge at 3000 revolutions per minute for 10 minutes to separate serum.

(vi) Serum calcium determination: Pipet 0.1 mL of the serum, add exactly 6.9 mL of strontium TS, mix well, and use this solution as the sample solution for calcium determination. Separately, pipet a suitable volume of Standard Calcium Solution for Atomic Absorption Spectrophotometry, dissolve in strontium TS to make a solution so that each mL contains 0.2 to 3 μg of calcium (Ca: 40.08), and use this solution as the standard solution for calcium determination. Perform the test as directed under Atomic Absorption Spectrometry <*2.23*> according to the following conditions, and calculate the calcium content of the sample solution for calcium determination from the calibration curve obtained from the absorbance of the standard solution for calcium determination.

Amount (mg) of Calcium (Ca) in 100 mL of the serum
= Calcium content (ppm) in the sample solution for calcium determination × 7

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Calcium hollow-cathode lamp
Wavelength: 422.7 nm

(vii) Calculation: Amounts of calcium in the serum obtained with S_H, S_L, T_H and T_L are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Units per mg of peptide = antilog $M \times (b/a) \times (1/c) \times 5$

$M = 0.3010 \times (Y_a/Y_b)$

$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$

$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

a: Amount (mg) of Calcitonin (Salmon)

b: Total volume (mL) of the high-dose sample solution prepared by dissolving Calcitonin (Salmon) in acetic acid buffer solution containing 0.1% bovine serum albumin.

c: Peptide content (%)

F' computed by the following equation should be smaller than F_1 shown in the table against n with which s^2 is calculated. Calculate L (P=0.95) by use of the following equation: L should be not more than 0.20. If F' exceeds F_1, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F_1 and L is not

more than 0.20.

$F' = (-Y_1 + Y_2 + Y_3 - Y_4)^2/4fs^2$
f: Number of the test animals of each group.
$s^2 = \{\Sigma y^2 - (Y/f)\}/n$
Σy^2: The sum of squares of y_1, y_2, y_3 and y_4 in each group.
$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4(f - 1)$
$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$
$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$
t^2: Value shown in the following table against n used to calculate s^2.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, not exceeding 10ºC.

Carmellose

カルメロース

Add the following next to the Japanese title:

[*9000-11-7*]

Carmellose Calcium

カルメロースカルシウム

Add the following next to the Japanese title:

[*9050-04-8*]

Carmellose Sodium

カルメロースナトリウム

Add the following next to the Japanese title:

[*9004-32-4*]

Cefaclor Compound Granules

セファクロル複合顆粒

Change the Purity to read:

Purity Related substances—Take out the total contents of not less than 5 packs of Cefaclor Compound Granules, add a small amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind, add 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, and add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly V mL so that each mL contains about 5 mg (potency) of Cefaclor according to the labeled total potency. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solutions, pH 4.5, to make exactly 20 mL. Pipet 2 mL of this solution, add 0.1 mol/L phosphate buffer solutions, pH 4.5, 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine each peak area of both solutions by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution, pH 4.5.

$$\text{Amount (\%) of each related substance} = W_S \times (A_T/A_S) \times (V/4) \times \{1/(C \times T)\}$$

$$\text{Total amount (\%) of the related substances} = W_S \times (\Sigma A_T/A_S) \times (V/4) \times \{1/(C \times T)\}$$

W_S: Amount [mg (potency)] of Cefaclor Reference Standard
A_T: Area of each peak other than cefaclor, solvent and excipient from the sample solution
ΣA_T: Total area of the peaks other than cefaclor, solvent and excipient from the sample solution
A_S: Peak area of cefaclor from the standard solution
C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack
T: Number (pack) of sample

Operating conditions—
Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.
System suitability—
Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 50 μL of this solution is equivalent to 3.5 to 6.5% of that of cefaclor from the standard solution.
System performance: When the procedure is run with 50 μL

of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40000, and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Add the following:

Cefalexin Capsules

セファレキシンカプセル

Cefalexin Capsules contain not less than 93.0% and not more than 107.0% of the labeled potency of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of preparation Prepare as directed under Capsules, with Cefalexin.

Identification Take out the contents of Cefalexin Capsules, to a quantity of the contents, equivalent to 70 mg (potency) of Cefalexin according to the labeled amount, add 25 mL of water, shake vigorously for 5 minutes, and filter. To 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 260 nm and 264 nm.

Water *<2.48>* Not more than 10.0% (0.2 g, volumetric titration, back titration)

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Open 1 capsule of Cefalexin Capsules, add 3*V*/5 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly *V* mL so that each mL contains about 1.25 mg (potency) of Cefalexin. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard in each solution.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)

$= W_S \times (Q_T/Q_S) \times (V/20)$

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

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15000)

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard substance 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 ratio of the peak area of cefalexin to that of the internal standard substance is not more than 1.0%.

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rates in 30 minutes of 125-mg (potency) capsules and in 60 minutes of 250-mg (potency) capsules are not less than 75% and 80%, respectively.

Start the test with 1 capsule of Cefalexin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V′* mL so that each mL contains about 22 μg (potency) of Cefalexin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin Reference Standard, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$)

$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

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

C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 capsule

Assay Take out the contents of not less than 20 capsules of Cefalexin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solu-

tion, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard in each solution.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= W_S \times (Q_T/Q_S) \times 5$

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

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15000)

Operating conditions—

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

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin 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, cefalexin 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 ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Cefalexin for Syrup

シロップ用セファレキシン

Cefalexin for Syrup is a preparation for syrup, which is dissolved or suspended before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of preparation Prepare as directed under Syrup, with Cefalexin.

Identification Dissolve a quantity of Cefalexin for Syrup, equivalent to 3 mg (potency) of Cefalexin according to the labeled amount, in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 260 nm and 264 nm.

Water <*2.48*> Not more than 5.0% (0.4 g, volumetric titration, back titration)

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Cefalexin for Syrup in single-unit containers meets the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Cefalexin for Syrup, add 3*V*/5 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly *V* mL so that each mL contains about 1 mg (potency) of Cefalexin, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= W_S \times (Q_T/Q_S) \times (V/20)$

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

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15000)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefalexin for Syrup is not less than 80%.

Start the test with an accurately weighed amount of Cefalexin, equivalent to about 0.25 g (potency) of Cefalexin for Syrup according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin Reference Standard, equivalent to

about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 1125$

W_S: Amount [mg (potency)] of Cefalexin Reference Standard
W_T: Amount (g) of Cefalexin for Syrup
C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 g

Assay Powder Cefalexin for Syrup, if necessary, and weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5, shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5, to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard in each solution.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= W_S \times (Q_T/Q_S) \times 5$

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

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15000)

Operating conditions—

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

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin 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, cefalexin 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 ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Cefatrizine Propylene Glycolate for Syrup

シロップ用セファトリジンプロピレングリコール

Cefatrizine Propylene Glycolate for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 90.0% and not more than 105.0% of the labeled potency of Cefatrizine ($C_{18}H_{18}N_6O_5S_2$: 462.50).

Method of preparation Prepare as directed under Syrup, with Cefatrizine Propylene Glycolate.

Identification Powder Cefatrizine Propylene Glycolate for Syrup, weigh a portion of the powder, equivalent to 10 mg (potency) of Cefatrizine Propylene Glycolate according to the labeled amount, and dissolve in 10 mL of water. To 2 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 225 nm and 229 nm, and between 266 nm and 271 nm.

pH <*2.54*> Take an amount of Cefatrizine Propylene Glycolate for Syrup, equivalent to 0.4 g (potency) of Cefatrizine Propylene Glycolate according to the labeled amount, and suspend in 10 mL of water: the pH of this suspension is between 4.0 and 6.0.

Purity Related substances—Use the sample solution obtained in the Assay 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 exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than cefatrizine obtained from the sample solution is not larger than the peak area of cefatrizine from the standard solution, and the total area of the peaks other than the peak of cefatrizine obtained from the sample solution is not larger than 2 times the peak area of cefatrizine 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 under Cefatrizine Propylene Glycolate.

Time span of measurement: About 2.5 times as long as the retention time of cefatrizine, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Cefatrizine Propylene Glycolate.

Test for required detectability: Pipet 2 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of cefatrizine obtained from 10 μL of this solution is equivalent to 15 to 25% of that of cefatrizine from the standard solution.

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 cefatrizine is not more than 2.0%.

Uniformity of dosage units *<6.02>* Cefatrizine Propylene Glycolate for Syrup in single-unit containers meets the requirement of the Mass variation test.

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefatrizine Propylene Glycolate for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Cefatrizine Propylene Glycolate for Syrup, equivalent to about 0.1 g (potency) of Cefatrizine Propylene Glycolate, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefatrizine Propylene Glycolate Reference Standard, equivalent to about 28 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas of cefatrizine, A_T and A_S, of both solution.

Dissolution rate (%) with respect to the labeled amount of cefatrizine ($C_{18}H_{18}N_6O_5S_2$)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 360$

W_S: Amount [mg (potency)] of Cefatrizine Propylene Glycolate Reference Standard
W_T: Amount (g) of Cefatrizine Propylene Glycolate for Syrup
C: Labeled amount [mg (potency)] of cefatrizine propylene glycolate ($C_{18}H_{18}N_6O_5S_2 \cdot C_3H_8O_2$) in 1 g

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay under Cefatrizine Propylene Glycolate.

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (17 in 12500) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of cefatrizine 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 and the symmetry factor of the peak of cefatrizine 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 cefatrizine is not more than 1.0%.

Assay Powder Cefatrizine Propylene Glycolate for Syrup, weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefatrizine Propylene Glycolate, dissolve in water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefatrizine Propylene Glycolate Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefatrizine Propylene Glycolate.

Amount [mg (potency)] of cefatrizine ($C_{18}H_{18}N_6O_5S_2$)
$= W_S \times (A_T/A_S) \times 5$

W_S: Amount [mg (potency)] of Cefatrizine Propylene Glycolate Reference Standard

Containers and storage Containers—Tight containers.

Add the following:

Cefixime Capsules

セフィキシムカプセル

Cefixime Capsules contain not less than 90.0% and not more than 105.0% of the labeled potency of cefixime ($C_{16}H_{15}N_5O_7S_2$: 453.45).

Method of preparation Prepare as directed under Capsules, with Cefixime.

Identification Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 70 mg (potency) of Cefixime according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and filter. Pipet 1 mL of the filtrate, and add 0.1 mol/L phosphate buffer solution, pH 7.0, to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 286 nm and 290 nm.

Purity Related substances—Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 0.1 g (potency) of Cefixime according to the la-

beled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than cefixime is not more than 1.0%, and the total amount of the peaks other than cefixime is not more than 2.5%.

Operating conditions—

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

Time span for measurement: Proceed as directed in the operating conditions in the Purity under Cefixime.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution, pH 7.0, 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 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10 mL. Confirm that the peak area of cefixime obtained from 10 μL of this solution is equivalent to 7 to 13% of that of cefixime from 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 factor of the peak of cefixime are not less than 4000 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 cefixime is not more than 2.0%.

Water <*2.48*> Not more than 12.0% (0.1 g of the contents, volumetric titration, direct titration)

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Cefixime Capsules, and to the contents and the capsule shells add $7V/10$ mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly V mL so that each mL contains about 1 mg (potency) of Cefixime. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime.

Amount [mg (potency)] of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= W_S \times (A_T/A_S) \times (V/20)$

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

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.5, as the dissolution medium, the dissolution rates in 60 minutes of 50-mg (potency) capsules and in 90 minutes of 100-mg (potency) capsules are not less than 80%, respectively.

Start the test with 1 capsule of Cefixime Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 56 μg (potency) of Cefixime according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime Reference Standard, equivalent to about 28 mg (potency), and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cefixime in each solution.

Dissolution rate (%) with respect to the labeled amount of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$

W_S: Amount [mg (potency)] of Cefixime Reference Standard
C: Labeled amount [mg (potency)] of Cefixime in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cefixime.

System suitability—

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 factor of the peak of cefixime are not less than 4000 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 cefixime is not more than 2.0%.

Assay Take out the contents of not less than 20 Cefixime Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefixime, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and shake for 30 minutes, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime Reference Standard, equivalent to about 20 mg (potency), dis-

solve in 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime.

Amount [mg (potency)] of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= W_S \times (A_T/A_S) \times 5$

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

Containers and storage Containers—Tight containers.

Add the following:

Cefroxadine for Syrup

シロップ用セフロキサジン

Cefroxadine for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of cefroxadine ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of preparation Prepare as directed under Syrup, with Cefroxadine Hydrate.

Identification Powder Cefroxadine for Syrup, if necessary. To a portion of the powder, equivalent to 2 mg (potency) of Cefroxadine Hydrate according to the labeled amount, add 100 mL of 0.001 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 267 nm and 271 nm.

Water <*2.48*> Not more than 4.5% (0.1 g, volumetric titration, direct titration).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Cefroxadine for Syrup in single-unit containers meets the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Cefroxadine for Syrup, add 4*V*/5 mL of a mixture of dilute acetic acid and phosphoric acid (500:1), shake well for 15 minutes, add exactly 5 mL of the internal standard solution per 50 mg (potency) of Cefroxadine Hydrate, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make *V* mL so that each mL contains 0.25 mg (potency) of Cefroxadine Hydrate. Filter this solution through a membrane filter with pore size of not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefroxadine Reference Standard, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution, add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefroxadine Hydrate.

Amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= W_S \times (Q_T/Q_S) \times (V/200)$

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.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefroxadine for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Cefroxadine for Syrup, equivalent to about 0.1 g (potency) of Cefroxadine Hydrate according to the labeled amount, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefroxadine Reference Standard, equivalent to about 22 mg (potency), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of water, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 267 nm.

Dissolution rate (%) with respect to the labeled amount of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 450$

W_S: Amount [mg (potency)] of Cefroxadine Reference Standard
W_T: Amount (g) of Cefroxadine for Syrup
C: Labeled amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$) in 1 g

Assay Powder Cefroxadine for Syrup, if necessary, weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of Cefroxadine Hydrate, add 160 mL of a mixture of dilute acetic acid and phosphoric acid (500:1), shake well for 15 minutes, add exactly 5 mL of the internal standard solution, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefroxadine Reference Standard, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution, add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefroxadine Hydrate.

Amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$)

$= W_S \times (Q_T/Q_S)$

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.

Containers and storage Containers—Tight containers.

Add the following:

Cefteram Pivoxil Tablets

セフテラム ピボキシル錠

Cefteram Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Method of preparation Prepare as directed under Tablets, with Cefteram Pivoxil.

Identification To a quantity of powdered Cefteram Pivoxil Tablets, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 262 nm and 266 nm.

Purity Related substances—To a quantity of powdered Cefteram Pivoxil Tablets, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add diluted acetonitrile (1 in 2) to make 100 mL. Disperse this solution with ultrasonic waves, filter, and use the filtrate as the sample solution. Pipet 1 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil, obtained from the sample solution is not larger than 1.75 times the peak area of cefteram pivoxil from the standard solution, and the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, obtained from the sample solution is not larger than 17/25 times the peak area of cefteram pivoxil from the standard solution. Furthermore, the total area of the peaks other than cefteram pivoxil from the sample solution is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil, after multiplying by the relative response factor, 0.74.relative response

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefteram Pivoxil.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefteram Pivoxil.

Water *<2.48>* Not more than 4.0% (a quantity equivalent to 0.2 g (potency) of powdered Cefteram Pivoxil Tablets, volumetric titration, direct titration).

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cefteram Pivoxil Tablets add exactly 5 mL of the internal standard solution per 50 mg (potency) of Cefteram Pivoxil, and add diluted acetonitrile (1 in 2) to make V mL so that each mL contains about 1 mg (potency) of Cefteram Pivoxil. Disperse this solution with ultrasonic waves, filter through a membrane filter with pore size of not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= W_S \times (Q_T/Q_S) \times (V/50)$

W_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000)

Dissolution *<6.10>* When the test is performed at 75 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cefteram Pivoxil Tablets is not less than 75%.

Start the test with 1 tablet of Cefteram Pivoxil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefteram Pivoxil according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard, equivalent to about 22 mg (potency), and dissolve in 20 mL of methanol, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank, and determine the absorbances, A_T and A_S, at 300 nm.

Dissolution rate (%) with respect to the labeled amount of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

W_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard
C: Labeled amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$) in 1 tablet

Assay To a number of tablet of Cefteram Pivoxil Tablets, equivalent to about 1.0 g (potency) of Cefteram Pivoxil, add 120 mL of diluted acetonitrile (1 in 2), disperse with ultrasonic waves, and add diluted acetonitrile (1 in 2) to make exactly 200 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, filter through a membrane filter with pore size not exceeding 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= W_S \times (Q_T/Q_S) \times 20$

W_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000)

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cellacefate

セラセフェート

Change the Chemical name to read:

[*9004-38-0*]

Microcrystalline Cellulose

結晶セルロース

Add the following next to the Japanese title:

[*9004-34-6*, Cellulose]

Powdered Cellulose

粉末セルロース

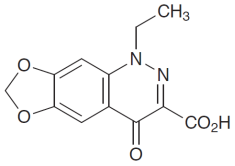
Add the following next to the Japanese title:

[*9004-34-6*, Cellulose]

Add the following:

Cinoxacin

シノキサシン



$C_{12}H_{10}N_2O_5$: 262.22
5-Ethyl-8-oxo-5,8-dihydro[1,3]dioxolo[4,5-*g*]cinnoline-7-carboxylic acid [*28657-80-9*]

Cinoxacin, when dried, contains not less than 98.0% and not more than 101.0% of $C_{12}H_{10}N_2O_5$.

Description Cinoxacin occurs as a white to pale yellow crystalline powder. It is odorless or has a slight, characteristic odor. It has a bitter taste.

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

It dissolves in sodium hydroxide TS.

Melting point: about 265ºC (with decomposition).

Identification (1) Dissolve 30 mg of Cinoxacin in 10 mL of dilute sodium hydroxide TS, and add water to make 100 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Cinoxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Sulfate <*1.14*>—Dissolve 0.20 g of Cinoxacin in 10 mL of dilute sodium hydroxide TS, add 20 mL of 0.1 mol/L hydrochloric acid TS, shake, filter, and add water to the filtrate to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.005 mol/L sulfuric acid by adding 10 mL of dilute sodium hydroxide TS, 20 mL of 0.1 mol/L hydrochloric acid TS, and water to make 50 mL (not more than 0.048%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cinoxacin

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 Cinoxacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of this 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 Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (14:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, 105ºC, 1 hour).

Residue on ignition *<2.44>* Not more than 0.2% (1 g).

Assay Weigh accurately about 0.4 g of Cinoxacin, previously dried, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and dissolve by warming. After cooling, titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.22 mg of $C_{12}H_{10}N_2O_5$

Containers and storage Containers—Tight containers.

Add the following:

Cinoxacin Capsules

シノキサシンカプセル

Cinoxacin Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of cinoxacin ($C_{12}H_{10}N_2O_5$: 262.22).

Method of preparation Prepare as directed under Capsules, with Cinoxacin.

Identification To a quantity of the contents of Cinoxacin Capsules, equivalent to 10 mg of Cinoxacin according to the labeled amount, add 20 mL of acetone, shake well, and centrifuge. To 3 mL of the supernatant liquid add acetone to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of cinoxacin for assay in 20 mL of acetone. To 3 mL of this solution add acetone to make 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (14:4:1) 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 and the spot from the standard solution show a blue-purple color and the same R_f value.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Cinoxacin Capsules add 40 mL of dilute sodium hydroxide TS, and dissolve the capsule in lukewarm water with occasional shaking. After cooling, add water and shake well, add water to make exactly V mL so that each mL contains about 1 mg of cinoxacin ($C_{12}H_{10}N_2O_5$), and filter. Discard the first 20 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of cinoxacin for assay, previously dried at 105ºC for 1 hour, dissolve in 40 mL of dilute sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 1 mL of this solution, add 0.1 mol/L of hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 354 nm.

Amount (mg) of cinoxacin ($C_{12}H_{10}N_2O_5$)
$= W_S \times (A_T/A_S) \times (V/200)$

W_S: Amount (mg) of cinoxacin for assay

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd solution for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Cinoxacin Capsules is not less than 70%.

Start the test with 1 capsule of Cinoxacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of cinoxacin ($C_{12}H_{10}N_2O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of cinoxacin for assay, previously dried at 105ºC for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 351 nm.

Dissolution rate (%) with respect to the labeled amount of cinoxacin ($C_{12}H_{10}N_2O_5$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$

W_S: Amount (mg) of cinoxacin for assay

C: Labeled amount (mg) of cinoxacin ($C_{12}H_{10}N_2O_5$) in 1 capsule

Assay Weigh accurately the mass of not less than 20 Cinoxacin Capsules, take out the contents, and powder. Wash the capsule shells with a small amount of diethyl ether, allow to stand at room temperature to vaporize the diethyl ether, weigh accurately the mass of the capsule shells, and calculate the mass of the contents. Weigh accurately a portion of the powder, equivalent to about 50 mg of cinoxacin ($C_{12}H_{10}N_2O_5$), add 10 mL of dilute sodium hydroxide TS, shake, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of cinoxacin for assay, previously dried at 105ºC for 1 hour, dissolve in 10 mL of dilute sodium hydroxide TS, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 354 nm.

$$\text{Amount (mg) of cinoxacin } (C_{12}H_{10}N_2O_5) = W_S \times (A_T/A_S)$$

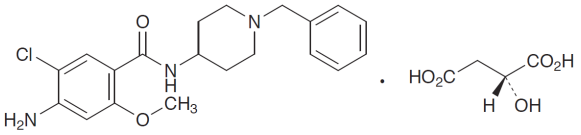
W_S: Amount (mg) of cinoxacin for assay

Containers and storage Containers—Well-closed containers.

Add the following:

Clebopride Malate

クレボプリドリンゴ酸塩



and enantiomer

$C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$: 507.96
4-Amino-*N*-(1-benzylpiperidin-4-yl)-5-chloro-2-methoxybenzamide mono-(2*RS*)-malate
[*57645-91-7*]

Clebopride Malate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$.

Description Clebopride Malate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

A solution of Clebopride Malate in methanol (1 in 25) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Clebopride Malate in methanol (1 in 80000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Clebopride Malate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clebopride Malate under Flame Coloration Test *<1.04>* (2): a green color appears.

Purity **(1)** Chloride *<1.03>*—Dissolve 1.0 g of Clebopride Malate in 20 mL of acetic acid (100), 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS by adding 20 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Clebopride Malate 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) Related substances—Dissolve 0.10 g of Clebopride Malate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.2 mL of this solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* 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 the peak of clebopride obtained from the sample solution is not larger than the peak area of clebopride 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 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: Dissolve 3.85 g of ammonium acetate in water to make 500 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. To 400 mL of the filtrate add 600 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of clebopride is about 15 minutes.

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

System suitability—

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

System performance: Dissolve 30 mg Clebopride Malate and 5 mg of propyl parahydroxybenzoate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and clebopride 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 clebopride is not more than 2.5%.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clebopride Malate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.80 mg of $C_{20}H_{24}ClN_3O_2 \cdot C_4H_6O_5$

Containers and storage Containers—Tight containers.

Clindamycin Hydrochloride

クリンダマイシン塩酸塩

Change the Origin/limits of content and Assay to read:

Clindamycin Hydrochloride is the hydrochloride of a derivative of lincomycin.

It contains not less than 838 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Hydrochloride is expressed as mass (potency) of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Assay Weigh accurately an amount of Clindamycin Hydrochloride and an amount of Clindamycin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve each in the mobile phase to make exactly 20 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clindamycin in each solution.

Amount [μg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$)
$= W_S \times (A_T/A_S) \times 1000$

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

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 25ºC.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of clindamycin 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, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 6000 and not more than 1.5, 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 clindamycin is not more than 1.0%.

Clindamycin Hydrochloride Capsules

クリンダマイシン塩酸塩カプセル

Change the Origin/limits of content, Uniformity of dosage units and Assay to read:

Clindamycin Hydrochloride Capsules contains not less than 93.0% and not more than 107.0% of the labeled potency of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clindamycin Hydrochloride Capsules add a suitable amount of the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.75 mg (potency) of Clindamycin Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$)
$= W_S \times (A_T/A_S) \times (V/100)$

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

Assay Take out the contents of not less than 20 Clindamycin Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Clindamycin Hydro-

chloride, add the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 75 mg (potency) of Clindamycin Hydrochloride Reference Standard, dissolve in 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, A_T and A_S, of clindamycin in each solution.

Amount [mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$)
$= W_S \times (A_T/A_S)$

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

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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: To 0.05 mol/L of potassium dihydrogen phosphate TS add 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of clindamycin 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 number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 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 clindamycin is not more than 1.0%.

Clomifene Citrate Tablets

クロミフェンクエン酸塩錠

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clomifene Citrate Tablets add 10 mL of water, and shake until the tablets are disintegrated. To this solution add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly V mL so that each mL contains about 20 μg of clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$)
$= W_S \times (A_T/A_S) \times (V/100)$

W_S: Amount (mg) of Clomifene Citrate Reference Standard

Codeine Phosphate Tablets

コデインリン酸塩錠

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Codeine Phosphate Tablets add 3V/25 mL of water to disintegrate, add 2V/25 mL of diluted dilute sulfuric acid (1 in 20), and treat with ultrasonic waves for 10 minutes. To this solution add exactly 2V/25 mL of the internal standard solution, add water to make V mL so that each mL contains about 0.2 mg of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$), filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (separately, determine the water content <*2.48*> in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$)
$= W_S \times (Q_T/Q_S) \times (V/250) \times 1.023$

W_S: Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethylefurin hydrochloride (3 in 2000)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Codeine Phosphate Tablets is not less than 80%.

Start the test with 1 tablet of Codeine Phosphate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate for assay (separately, determine the water content <*2.48*> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solu-

tion as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of codeine in each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18 \times 1.023$

W_S: Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis
C: Labeled amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot ½H_2O$) in 1 tablet

Operating procedures—
Proceed as directed in the Assay.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of codeine are not less than 5000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of codeine is not more than 2.0%.

Corn Starch

トウモロコシデンプン

Change the Identification (1) to read:

Identification (1) Under a microscope <*5.01*>, using a mixture of water and glycerin (1:1), Corn Starch appears as either angular polyhedral granules of irregular sizes with diameters ranging from about 2 μm to about 23 μm or as rounded or spheroidal granules of irregular sizes with diameters ranging from about 25 μm to about 35 μm. The central hilum consists of a distinct cavity or two- to five-rayed cleft, and there are no concentric striations. Between orthogonally oriented polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

Add the following next to the Purity (3):

Purity ◆**(4)** Foreign matter—Under a microscope <*5.01*>, Corn Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.◆

Change to read:

Wood Creosote

木クレオソート

Wood Creosote is a mixture of phenols obtained from by using wood tar derived from dry distillation of stems and branches of various plants of genus *Pinus* (*Pinaceae*), genus *Cryptomeria* (*Taxodiaceae*), genus *Fagus* (*Fagaceae*), genus *Afzelia* (genus *Intsia*); (*Leguminosae*), genus *Shorea* (*Dipterocarpaceae*) or genus *Tectona* (*Verbenaceae*), followed by distillation and collection at 180 to 230°C, then further purification and then re-distillation.

Wood Creosote contains not less than 23.0% and not more than 35 % of guaiacol ($C_7H_8O_2$:124.14).

Description Wood Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor.
It is slightly soluble in water.
It is miscible with methanol or ethanol (99.5).
Its saturated solution is acidic.
It is highly refractive.
It gradually changes in color by light or by air.

Identification The sample solution for assay is used as the sample solution. Separately dissolve 0.1 g of phenol, *p*-cresol, guaiacol and 2-methoxy-4-methylphenol in methanol respectively, to prepare 100 mL of solutions. Take 10 mL of each solution and add methanol to prepare 50 mL of solutions, which are used as Standard Solution (1), Standard Solution (2), Standard Solution (3) and Standard Solution (4). The perform test with 10 μL each of the sample solution, Standard Solution (1), Standard Solution (2), Standard Solution (3) and Standard Solution (4) as directed under liquid chromatography <*2.01*> according to the following conditions: the main peaks show the same retention times with those obtained with the Standard Solution (1-4).
Operating conditions—
It meets test requirements directed in the assay.

Specific gravity <*2.56*> d_{20}^{20}: Not less than 1.076.

Purity (1) Coal Creosote Accurately measure 10 mL of this Coal Creosote, add methanol to prepare exactly 20 mL of the solution, which is used as the standard solution. Separately prepare exactly 1 mg each of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*, *h*]anthracene, dissolve ethyl acetate as needed and add methanol to prepare a 100 mL solution. Take 1 mL of this solution and add methanol to prepare 100 mL of the standard solution. Perform the test with exactly 1 μL each of sample solution and standard solution as directed under gas chromatography <*2.02*> according to the following conditions: No peaks are detected for retention times that correspond to benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*, *h*]anthracene. Change these conditions if any peak is detected for retention times that correspond to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*, *h*]anthracene, to verify that such

a peak does not belong to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*, *h*]anthracene.

Operating conditions—

Detector: Mass spectrometer (EI).

Monitored ions:

Benz[*a*]anthracene: Molecular ion *m/z* 228, Fragment ion *m/z* 114	About 14 to 20 minutes
Benzo[*a*]pyrene: Molecular ion *m/z* 252, Fragment ion *m/z* 125	About 20 to 25 minutes
Dibenz[*a*, *h*]anthracene: Molecular ion *m/z* 278, Fragment ion *m/z* 139	About 25 to 30 minutes

Column: A quartz tube 0.25 mm in inside diameter and 30 m in length, with internal coating 0.25 – 0.5 μm in thickness made of 5% diphenyl and 95% dimethyl polysiloxane for gas chromatography.

Column temperature: Perform injection at constant temperature in vicinity of 45°C, then raise temperature by 40°C each time to 240°C, maintain the temperature at 240°C for 5 minutes, then raise temperature by 4°C per minute until it reaches 300°C, then raise the temperature by 10°C each minute until it reaches 320°C, then maintain temperature at 320°C for 3 minutes.

Injection port temperature: Constant temperature in vicinity of 250°C.

Interface temperature: Constant temperature in vicinity of 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of benzo [*a*]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability—

Test for required deectability: Accurately measure 1 mL of standard solution and add methanol to prepare exactly 10 mL of the solution for system suitability test. When the test is performed with conditions described above for 1 μL of the solution for system suitability test, the S/N ratio of each substance is not less than 3.

System performance: When the procedure is run with conditions described above for 1 μL of the solution for system suitability test, the elution takes place in order of benz[*a*]anthracene, benzo[*a*]pyrene and then dibenz[*a*, *h*]anthracene.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*, *h*]anthracene is respectively not more than 10 %.

(2) Acenaphthene

Add methanol to 0.12 g of Wood Creosote to prepare 50 mL of the sample solution. Separately dissolve 25 mg of acenaphthene in methanol to prepare a 50 mL of solution. Take 5 mL of this solution and add methanol to prepare 20 mL of solution. Take 2 mL of this solution and add methanol to prepare 100 mL of standard solution. Perform the test with exactly 1 μL each of sample solution and standard solution as directed under gas chromatography <*2.02*> according to the following conditions: No peaks are detected with sample solution for the retention time corresponding to acenaphthene of the standard solution. Change these conditions if any peak is detected for the retention time corresponding to acenaphthene, to verify that such a peak does not belong to athenaphthene.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.25 mm inside diameter and 60 m in length, with internal coating 0.25 – 0.5 μm in thickness made of polymethylsiloxane for gas chromatography.

Column temperature: Perform injection at a constant temperature in vicinity of 45°C, then raise the temperature by 11.5°C per minute until reaching 160°C, then raise the temperature by 4°C per minute until reaching 180°C, then raise the temperature by 8°C until reaching 270°C, then maintain temperature at 270°C for 3 minutes.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of acenaphthene is about 18 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of this solution, add methanol to prepare exactly 10 mL of the solution for system suitability test. When the procedure is run with conditions described above for 1 μL of solution for system suitability test, the S/N ratio of acenaphthene is not less than 3.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acenaphthene is not more than 6.0 %.

(3) Other impurities

Add 2 mL of petroleum benzin to 1.0 mL of Wood Creosote, then add 2 mL of barium hydroxide test solution, agitate to mix and allow to stand. No blue or muddy brown color develops in the upper layer of the mixture. Furthermore, no red color develops in the lower layer.

Distilling range <*2.57*> 200 – 220°C, not less than 85 vol%.

Assay Add methanol to exactly 0.1 g of Wood Creosote to prepare exactly 50 mL of the solution. Add methanol to exactly 10 mL of this solution to prepare 50 mL of sample solution. Separately add methanol to about 30 mg of accurately measured guaiacol for assay to prepare exactly 50 mL of the solution. Accurately measure 10 mL of this solution, add methanol to prepare exactly 50 mL of the standard solution. Perform the test with 10 μL each of sample solution and standard solution as directed under liquid chromatography <*2.01*> according to the following conditions, and calculate the peak areas, A_T and A_S, of the guaiacol for each solution.

The amount of guaiacol ($C_7H_8O_2$) (mg)
$= W_S \times (A_T / A_S)$

W_S: Amount of guaiacol for assay (mg)

Operating conditions—

Detector: Ultraviolet absorption detector (detected wavelength: 275 nm).

Column: Fill a stainless steel tube with internal diameter of 4.6 mm and length of 15 cm with 5 μm of octadecylsilanized silica gel for gas chromatography.

Column temperature: Constant temperature in vicinity of 40°C.

Mobile phase: Mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of guaiacol is about 9 minutes.

System suitability—

System performance: Dissolve 2 mg each of guaiacol and phenol to obtain 10 mL of solution. The procedure is run with conditions described above for 10 μL of this solution, the elution takes place in order of phenol then guaiacol, with the degree in separation of 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 guaiacol is not more than 1.5 %.

Containers and storage Containers— Tight containers.

Storage—Light-resistant.

Croscarmellose Sodium

クロスカルメロースナトリウム

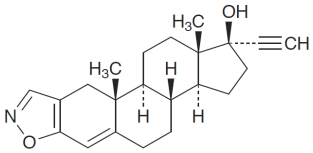
Add the following next to the Japanese title:

[*74811-65-7*]

Add the following:

Danazol

ダナゾール



$C_{22}H_{27}NO_2$: 337.46

17*α*-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol

[*17230-88-5*]

Danazol, when dried, contains not less than 98.5% and not more than 101.0% of $C_{22}H_{27}NO_2$.

Description Danazol occurs as a white to pale yellow crystalline powder.

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

Melting point: about 225ºC (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Danazol in ethanol (95) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Danazol 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 Danazol as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Danazol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation *<2.49>* $[\alpha]_D^{20}$: +8 - +11º (after drying, 0.25 g, ethanol (99.5), 50 mL, 100 mm).

Purity (1) Chloride *<1.03>*—To 2.0 g of Danazol add 80 mL of water, shake well, and boil for 5 minutes. After cooling, add water to make 100 mL, and filter through a glass filter (G4). Discard the first 30 mL of the filtrate, take 40 mL of the subsequent filtrate, 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Danazol 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) Related substances—Dissolve 0.20 g of Danazol in 4 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of this solution, add acetone to make exactly 200 mL. Pipet 4 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) 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 other than the spot at the starting point obtained from the sample solution are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.2% (1 g, in vacuum, phosphorous (V) oxide, 60ºC, 4 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Danazol and Danazol Reference Standard, previously dried, dissolve separately in ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 285 nm.

Amount (mg) of danazol ($C_{22}H_{27}NO_2$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Danazol Reference Standard

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Add the following:

Dibekacin Sulfate Ophthalmic Solution

ジベカシン硫酸塩点眼液

Dibekacin Sulfate Ophthalmic Solution is an aqueous ophthalmic solution.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52).

Method of preparation Prepare as directed under Ophthalmic Solution, with Dibekacin Sulfate.

Description Dibekacin Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification To a volume of Dibekacin Sulfate Ophthalmic Solution add water so that each mL contains about 2.5 mg (potency) of Dibekacin Sulfate according to the labeled amount, and use this solution as the sample solution. Separately, dissolve an amount of Dibekacin Sulfate Reference Standard, equivalent to 5 mg (potency), in 2 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Proceed as directed in the Identification (1) under Dibekacin Sulfate.

pH <*2.54*> 6.5 - 7.5

Foreign insoluble matter <*6.11*> It meets the requirement.

Insoluble particulate matter <*6.08*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Dibekacin Sulfate.

(ii) Sample solutions—Pipet a volume of Dibekacin Sulfate Ophthalmic Solution, equivalent to about 12 mg (potency), and add water to make exactly 30 mL. Pipet a suitable volume 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 low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Diethylcarbamazine Citrate Tablets

ジエチルカルバマジンクエン酸塩錠

Change the Origin/limits of content and Identification to read:

Diethylcarbamazine Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O{\cdot}C_6H_8O_7$: 391.42).

Identification To a quantity of the powdered Diethylcarbamazine Citrate Tablets, equivalent to 0.1 g of Diethylcarbamazine Citrate according to the labeled amount, add 10 mL of water, shake well, and filter. To the filtrate add 1 mL of Reinecke salt TS: a light red precipitate is formed.

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Diethylcarbamazine Citrate Tablets add 70 mL of the mobile phase, shake vigorously for 10 minutes, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to about 2.5 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O{\cdot}C_6H_8O_7$), add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O{\cdot}C_6H_8O_7$)
$= W_S \times (Q_T/Q_S) \times (10/V)$

W_S: Amount (mg) of Diethylcarbamazine Citrate Reference Standard

Internal standard solution—A solution of 2-aminobenzimidazol in the mobile phase (1 in 12500)

Change the Assay to read:

Assay Weigh accurately the mass of not less than 20 Diethylcarbamazine Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O{\cdot}C_6H_8O_7$), add 70 mL of the mobile phase, shake vigorously for 10 minutes, add the mobile phase to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the

filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Diethylcarbamazine Citrate Reference Standard, previously dried at 105ºC 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, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of diethylcarbamazine to that of the internal standard.

Amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$)
$= W_S \times (Q_T/Q_S) \times 2$

W_S: Amount (mg) of Diethylcarbamazine Citrate Reference Standard

Internal standard solution—A solution of 2-aminobenzimidazole in the mobile phase (1 in 12500)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 40ºC.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 2.5. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of diethylcarbamazine is about 14 minutes.

System suitability—

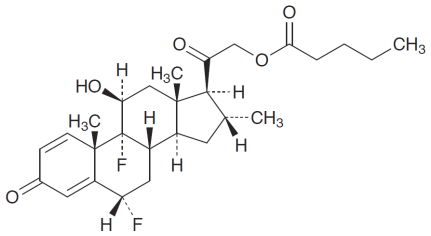
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, diethylcarbamazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

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 ratio of the peak area of diethylcarbamazine to that of the internal standard is not more than 1.0%.

Add the following:

Diflucortolone Valerate

ジフルコルトロン吉草酸エステル



$C_{27}H_{36}F_2O_5$: 478.57

6α,9-Difluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pentanate

[*59198-70-8*]

Diflucortolone Valerate contains not less than 98.0% and not more than 102.0% of $C_{27}H_{36}F_2O_5$, calculated on the dried basis.

Description Diflucortolone Valerate occurs as white crystals or crystalline powder.

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

Identification **(1)** Prepare the test solution by proceeding with 10 mg of Diflucortolone Valerate according to the Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> for fluoride.

(2) Determine the absorption spectrum of a solution of Diflucortolone Valerate in methanol (3 in 200000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Diflucortolone Valerate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Diflucortolone Valerate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Diflucortolone Valerate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +110 - +115º (0.1 g calculated on the dried basis, ethanol (99.5), 10 mL, 100 mm).

Melting point <*2.60*> 200 - 204ºC

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Diflucortolone Valerate in a platinum crucible 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). Carbonize and incinerate as directed under Residue on Ignition

<2.44>.

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method, and calculate the amounts of these peaks by the area percentage method: the amount of each peak of flucortolone valerate, 12α diflucortolone valerate and Δ4 diflucortolone valerate, having the relative retention times of about 0.97, 1.03 and 1.05 with respect to diflucortolone valerate, respectively, is not more than 0.6%, respectively; the amount of the peak of clocortolone valerate, having the relative retention time of about 1.09 with respect to diflucortolone valerate, is not more than 0.3%; and the amount of each peak other than those mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than the peak of diflucortolone valerate is not more than 2.0%.

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 1.4 times as long as the retention time of diflucortolone valerate, beginning 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: Pipet 0.1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 10 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 (1:1) to make exactly 20 mL. Confirm that the peak area of diflucortolone valerate obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that of diflucortolone valerate from the solution for system suitability test.

(3) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, 105ºC, 3 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 5 mg each of Diflucortolone Valerate and Diflucortolone Valerate Reference Standard (separately, determine the loss on drying *<2.41>* in the same manner as Diflucortolone Valerate), dissolve each in a mixture of water and acetonitrile (1:1) to make exactly 10 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of diflucortolone valerate in each solution.

Amount (mg) of diflucortolone valerate ($C_{27}H_{36}F_2O_5$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Diflucortolone Valerate Reference Standard, calculated on dried basis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

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

Column temperature: A constant temperature of about 25ºC.

Mobile phase A: To 0.02 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. Mix this solution with acetonitrile for liquid chromatography (11:9).

Mobile phase B: 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 (vol%)	Mobile phase B (vol%)
0 – 10	100 → 90	0 → 10
10 – 25	90	10
25 – 45	90 → 35	10 → 65
45 – 50	35	65

Flow rate: 1.0 mL per minute.

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 factor of the peak of diflucortolone valerate are not less than 10000 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 diflucortolone valerate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Digoxin

ジゴキシン

Change the System suitability in the Purity (2) Related substances to read:

Purity (2) Related substances

System suitability—

Test for required detectability: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 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 dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of this solution is equivalent to 0.07 to 0.13% of that from the solution for system suitability test.

System performance: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Digoxin Injection

ジゴキシン注射液

Change the Method of preparation to read:

Method of preparation Prepare as directed under Injections, with a solution of Digoxin in 10 to 50 vol% ethanol.

Add the following next to the Identification:

Alcohol number <*1.01*> 0.8 - 1.2 (Method 1).

Purity Related substances—To a volume of Digoxin Injection, equivalent to 2.5 mg of Digoxin according to the labeled amount, add dilute ethanol 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 Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of these peaks: the total amount of the peaks other than digoxin is not more than 5%.

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 digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 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 dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL this solution is equivalent to 0.07 to 0.13% of that of digoxin from the solution for system suitability test.

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Digoxin Tablets

ジゴキシン錠

Add the following next to the Identification:

Purity Related substances—Powder not less than 20 Digoxin Tablets. Weigh a portion of the powder equivalent to 2.5 mg of Digoxin according to the labeled amount, add 30 mL of dilute ethanol, treat with ultrasonic waves for 20 minutes, and shake for 5 minutes. After cooling, add dilute ethanol to make 50 mL, filter, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amount of these peaks by the area percentage method: the total amount of the peaks other than digoxin is not more than 5%.

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 digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 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 dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained from 10 μL of this solution is equivalent to 0.07 to 0.13% of that of digoxin from the solution for system suitability test.

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate 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 solution for system suitability test under the above

operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Dimenhydrinate Tablets

ジメンヒドリナート錠

Add the following next to the Identification:

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Dimenhydrinate Tablets is not less than 85%.

Start the test with 1 tablet of Dimenhydrinate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of dimenhydrinate for assay, previously dried in vacuum over phosphorous (V) oxide for 24 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Dissolution rate (%) with respect to the labeled amount of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

W_S: Amount (mg) of dimenhydrinate for assay
C: Labeled amount (mg) of dimenhydrinate ($C_{17}H_{21}NO{\cdot}C_7H_7ClN_4O_2$) in 1 tablet

Distigmine Bromide Tablets

ジスチグミン臭化物錠

Add the following next to the Identification:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Distigmine Bromide Tablets add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, and add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 30 μg of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$)
$= W_S \times \{(A_{T2} - A_{T1}) / (A_{S2} - A_{S1})\} \times (V'/V) \times (1/20)$

W_S: Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis

Dissolution *<6.10>* When the test is performed at 75 revolutions per minute according to the Paddle method using 500 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Distigmine Bromide Tablets is not less than 80%.

Start the test with 1 tablet of Distigmine Bromide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of distigmine bromide for assay (separately, determine the water content *<2.48>* in the same manner as Distigmine Bromide), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 500 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_{T1} and A_{S1}, at 270 nm, and A_{T2} and A_{S2}, at 350 nm.

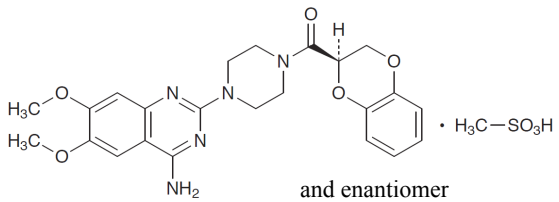
Dissolution rate (%) with respect to the labeled amount of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$)
$= W_S \times \{(A_{T1} - A_{T2}) / (A_{S1} - A_{S2})\} \times (V'/V) \times (1/C) \times 10$

W_S: Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis
C: Labeled amount (mg) of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$) in 1 tablet

Add the following:

Doxazosin Mesilate

ドキサゾシンメシル酸塩



and enantiomer

$C_{23}H_{25}N_5O_5{\cdot}CH_4O_3S$: 547.58
1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-4-{[(2*RS*)-2,3-dihydro-1,4-benzodioxin-2-yl]carbonyl}piperazine monomethansulfonate [*77883-43-3*]

Doxazosin Mesilate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{23}H_{25}N_5O_5{\cdot}CH_4O_3S$.

Description Doxazosin Mesilate occurs as a white to yellow-

ish white crystalline powder.

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

A solution of Doxazosin Mesilate in dimethylsulfoxide solution (1 in 20) shows no optical rotation.

Melting point: about 272ºC (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Doxazosin Mesilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxazosin Mesilate 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 Doxazosin Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum or the spectrum of Doxazosin Mesilate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) 30 mg of Doxazosin Mesilate responds to the Qualitative Tests *<1.09>* (2) for mesilate.

Purity **(1)** Heavy metals *<1.07>*—Proceed with 1.0 g of Doxazosin Mesilate 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 20 mg of Doxazosin Mesilate in 5 mL of a mixture of methanol and acetic acid (100) (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of methanol and acetic acid (100) (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with an upper layer of a mixture, prepared by adding 1 volume of water and 1 volume of acetic acid (100) to 2 volumes of 4-methyl-2-pentanon and shaking, to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot at the R_f value of about 0.15 obtained from the sample solution is not more intense than the spot from the standard solution, and no spots other than the principal spot and other than the spots mentioned above appear from the sample solution.

(3) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 1.0% (1 g, 105ºC, 4 hours).

Residue on ignition *<2.44>* Not more than 0.2% (1 g).

Assay Weigh accurately about 25 mg each of Doxazosin Mesilate and Doxazosin Mesilate Reference Standard, previously dried, dissolve separately in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of doxazosin in each solution.

Amount (mg) of doxazosin mesilate ($C_{23}H_{25}N_5O_5 \cdot CH_4O_3S$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Doxazosin Mesilate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 246 nm)

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and acetonitrile (12:8:3).

Flow rate: Adjust the flow rate so that the retention time of doxazosin 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, the number of theoretical plates and the symmetry factor of the peak of doxazosin are not less than 2000 and not more than 2.0, respectively.

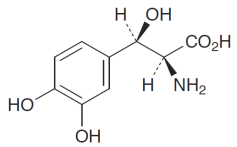
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxazosin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Droxidopa

ドロキシドパ



$C_9H_{11}NO_5$: 213.19
(2*S*,3*R*)-2-Amino-3-(3,4-dihydroxyphenyl)-3-hydroxypropanoic acid [*23651-95-8*]

Droxidopa, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_{11}NO_5$.

Description Droxidopa occurs as white to light brown crystals or crystalline powder.

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

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Droxidopa in 0.1 mol/L hydrochloric acid TS (1 in 25000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Droxidopa as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation *<2.49>* $[\alpha]_D^{20}$: –38 - –43º (after drying, 0.1 g, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

Purity (1) Chloride *<1.03>*—Dissolve 0.40 g of Droxidopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals *<1.07>*—Proceed with 2.0 g of Droxidopa 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 *<1.11>*—Prepare the test solution with 1.0 g of Droxidopa according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Droxidopa add 50 mL of 0.1 mol/L hydrochloric acid TS, dissolve by shaking while cooling in an ice bath, and use this solution as the sample solution. Pipet 1 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than droxidopa obtained from the sample solution is not larger than the peak area of droxidopa from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 (3 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.0 with phosphoric acid. To 930 mL of this solution add 70 mL of acetonitrile.

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

Time span of measurement: About 12 times as long as the retention time of droxidopa, beginning after the solvent peak.

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 factor of the peak of droxidopa are not less than 10000 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 droxidopa is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.1% (1 g, in vacuum, 60ºC, 3 hours).

Residue on ignition *<2.44>* Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Droxidopa, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS, add 50 mL of acetic acid (100), and titrate *<2.50>* the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.32 mg of $C_9H_{11}NO_5$

Containers and storage Containers—Well-closed containers.

Add the following:

Droxidopa Capsules

ドロキシドパカプセル

Droxidopa Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of droxidopa ($C_9H_{11}NO_5$: 213.19).

Method of preparation Prepare as directed under Capsules, with Droxidopa.

Identification (1) To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of water, shake for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat in a water bath for 3 minutes: a blue-purple color develops.

(2) To an amount of the contents of Droxidopa Capsules, equivalent to 20 mg of Droxidopa according to the labeled amount, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.

(3) To an amount of the contents of Droxidopa Capsules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and

filter. Discard the first 10 mL of the filtrate, and to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 278 nm and 282 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To the contents of 1 capsule of Droxidopa Capsules, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of droxidopa ($C_9H_{11}NO_5$). Filter this solution, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60ºC for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 280 nm.

Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= W_S \times (A_T/A_S) \times (V/100)$

W_S: Amount (mg) of droxidopa for assay

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Droxidopa Capsules is not less than 70%.

Start the test with 1 capsule of Droxidopa Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of droxidopa ($C_9H_{11}NO_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60ºC for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_{T1} and A_{S1}, at 280 nm, and A_{T2} and A_{S2}, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of droxidopa ($C_9H_{11}NO_5$)
$= W_S \times \{(A_{T1} - A_{T2}) / (A_{S1} - A_{S2})\} \times (V'/V) \times (1/C) \times 180$

W_S: Amount (mg) of droxidopa for assay
C: Labeled amount (mg) of droxidopa ($C_9H_{11}NO_5$) in 1 capsule

Assay Take out the contents of not less than 20 Droxidopa Capsules, weigh accurately the mass of the contents, and mix uniformly. Weigh accurately an amount equivalent to about 50 mg of droxidopa ($C_9H_{11}NO_5$), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60ºC for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 280 nm.

Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of droxidopa for assay

Containers and storage Containers—Tight containers.

Add the following:

Droxidopa Fine Granules

ドロキシドパ細粒

Droxidopa Fine Granules contain not less than 93.0% and not more than 107.0% of droxidopa ($C_9H_{11}NO_5$: 213.19).

Method of preparation Prepare fine granules as directed under Powders, with Droxidopa.

Identification **(1)** To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of water, shake for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, heat in a water bath for 3 minutes: a blue-purple color develops.

(2) To a quantity of powdered Droxidopa Fine Granules, equivalent to 20 mg of Droxidopa according to the labeled amount, add 20 mL of diluted acetic acid (100) (1 in 500), shake for 10 minutes, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to light brown.

(3) To a quantity of powdered Droxidopa Fine Granules, equivalent to 50 mg of Droxidopa according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. Discard the first 10 mL of the filtrate, to 2 mL of the subsequent filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 278 nm and 282 nm.

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Droxidopa Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Droxidopa Fine Granules, equivalent to about 0.1 g of droxidopa ($C_9H_{11}NO_5$), according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of water, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of droxidopa for assay, previously dried in vacuum at 60ºC for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_{T1} and A_{S1}, at 280 nm, and A_{T2} and A_{S2}, at 350 nm.

Dissolution rate (%) with respect to the labeled amount of droxidopa ($C_9H_{11}NO_5$)
$= (W_S/W_T) \times \{(A_{T1} - A_{T2}) / (A_{S1} - A_{S2})\} \times (1/C) \times 360$

W_S: Amount (mg) of droxidopa for assay
W_T: Amount (g) of Droxidopa Fine Granules
C: Labeled amount (mg) of droxidopa ($C_9H_{11}NO_5$) in 1 g

Particle size <*6.03*> It meets the requirement.

Assay Powder not less than 20 g of Droxidopa Fine Granules. Weigh accurately a portion of the powder, equivalent to about 50 mg of droxidopa ($C_9H_{11}NO_5$), add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of droxidopa for assay, previously dried in vacuum at 60ºC for 3 hours, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 280 nm.

Amount (mg) of droxidopa ($C_9H_{11}NO_5$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of droxidopa for assay

Containers and storage Containers—Tight containers.

Add the following:

Ecabet Sodium Granules

エカベトナトリウム顆粒

Ecabet Sodium Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$: 492.56).

Method of preparation Prepare as directed under Granules, with Ecabet Sodium Hydrate.

Identification To a quantity of Ecabet Sodium Granules, equivalent to 50 mg of Ecabet Sodium Hydrate according to the labeled amount, add 25 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, and to 3 mL of the subsequent filtrate add dilute sodium hydroxide TS to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 269 nm and 273 nm, and between 278 nm and 282 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Ecabet Sodium Granules in single-unit containers meets the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Ecabet Sodium Granules, add 70 mL of dilute sodium hydroxide TS, treat with ultrasonic waves for 5 minutes with occasional shaking, add dilute sodium hydroxide TS to make exactly V mL so that each mL contains about 10 mg of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$), and filter. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ecabet sodium hydrate for assay (separately, determine the water content <*2.48*> in the same manner as Ecabet Sodium Hydrate), dissolve in 2 mL of dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 271 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank

Amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$)
$= W_S \times (A_T/A_S) \times (V/2) \times 1.224$

W_S: Amount (mg) of ecabet sodium hydrate for assay, calculated on the anhydrous basis.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ecabet Sodium Granules is not less than 80%.

Start the test with an accurately weighed amount of Ecabet Sodium Granules, equivalent to about 1 g (potency) of Ecabet Sodium Hydrate according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the fil-

trate, pipet 2 mL of the subsequent filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of ecabet sodium hydrate for assay (separately, determine the water content *<2.48>* in the same manner as Ecabet Sodium Hydrate), dissolve in 1 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 271 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank

Dissolution rate (%) with respect to the labeled amount of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$)
$= (W_S / W_T) \times (A_T/A_S) \times (1/C) \times 4500 \times 1.224$

W_S: Amount (mg) of ecabet sodium hydrate for assay, calculated on the anhydrous basis
W_T: Amount (g) of Ecabet Sodium Granules
C: Labeled amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$) in 1 g

Particle size *<6.03>* It meets the requirement.

Assay Weigh accurately an amount of Ecabet Sodium Granules, equivalent to about 30 mg of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$), add exactly 5 mL of the internal standard solution, add 25 mL of diluted methanol (1 in 2), shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 3 mL of the subsequent filtrate add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ecabet sodium hydrate for assay (separately, determine the water content *<2.48>* in the same manner as Ecabet Sodium Hydrate), add exactly 5 mL of the internal standard solution, and dissolve in dilute methanol (1 in 2) to make 30 mL. To 3 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ecabet to that of the internal standard.

Amount (mg) of ecabet sodium hydrate ($C_{20}H_{27}NaO_5S \cdot 5H_2O$)
$= W_S \times (Q_T/Q_S) \times 1.224$

W_S: Amount (mg) of ecabet sodium hydrate for assay, calculated on the anhydrous basis

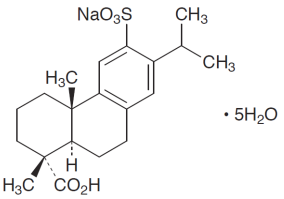
Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (1 in 2) (3 in 400)
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 225 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40ºC.
Mobile phase: To 0.1 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. To 730 mL of this solution add 270 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of ecabet 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, ecabet 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ecabet to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Ecabet Sodium Hydrate

エカベトナトリウム水和物



$C_{20}H_{27}NaO_5S \cdot 5H_2O$: 492.56
(1*R*,4a*S*,10a*S*)-1,4a-Dimethyl-7-(1-methylethyl)-6-sodiosulfonato-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid pentahydrate
[*219773-47-4*]

Ecabet Sodium Hydrate contains not less than 98.5% and not more than 101.5% of ecabet sodium ($C_{20}H_{27}NaO_5S$: 402.48), calculated on the anhydrous basis.

Description Ecabet Sodium Hydrate is white crystals.
It is freely soluble in methanol, and slightly soluble in water and in ethanol (99.5).
It dissolves in sodium hydroxide TS.
Dissolve 1.0 g of Ecabet Sodium Hydrate in 200 mL of water: the pH of the solution is about 3.5.

Identification **(1)** Determine the absorption spectrum of a solution of Ecabet Sodium Hydrate in dilute sodium hydroxide TS (3 in 10000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Ecabet Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Place 1 g of Ecabet Sodium Hydrate in a porcelain crucible, and carbonize. After cooling, add 0.5 mL of nitric acid, heat gradually to incinerate, and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +69 - +76º (0.25 g calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Ecabet Sodium Hydrate 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 10 mg of Ecabet Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than ecabet obtained from the sample solution is not larger than the peak area of ecabet from the standard solution.

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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: To 0.1 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust the pH to 3.0. To 730 mL of this solution add 270 mL of acetonitrile.

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

Time span of measurement: About 2 times as long as the retention time of ecabet, beginning after the solvent peak.

System suitability—

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 factor of the peak of ecabet are not less than 5000 and not more than 1.5, 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 ecabet is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> 17.3 - 19.2% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 1.2 g of Ecabet Sodium Hydrate, dissolve in 30 mL of methanol, add 30 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{20}H_{27}NaO_5S$

Containers and storage Containers—Well-closed containers.

Add the following:

Emorfazone Tablets

エモルファゾン錠

Emorfazone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of emorfazone ($C_{11}H_{17}N_3O_3$: 239.27).

Method of preparation Prepare as directed under Tablets, with Emorfazone.

Identification To a quantity of powdered Emorfazone Tablets, equivalent to 0.1 g of Emorfazone according to the labeled amount, add 100 mL of water, shake well, and centrifuge. Filter the supernatant liquid, and to 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 237 nm and 241 nm, and between 310 nm and 314 nm, and a shoulder between 288 nm and 298 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Emorfazone Tablets add methanol to make exactly V mL so that each mL contains about 4 mg of emorfazone ($C_{11}H_{17}N_3O_3$), and shake well to disintegrate. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$)
$= W_S \times (Q_T/Q_S) \times (V/5)$

W_S: Amount (mg) of emorfazone for assay

Internal standard solution—A solution of 2,4-dinitrophenylhidrazine in methanol (3 in 2000). Prepare before use.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Emorfazone Tablets is not less than 80%.

Start the test with 1 tablet of Emorfazone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make

exactly V' mL so that each mL contains about 11 μg of emorfazone ($C_{11}H_{17}N_3O_3$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of emorfazone for assay, previously dried in vacuum at 60ºC for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>

Dissolution rate (%) with respect to the labeled amount of emorfazone ($C_{11}H_{17}N_3O_3$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of emorfazone for assay
C: Labeled amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$) in 1 tablet

Assay To 10 tablets of Emorfazone Tablets add 200 mL of methanol, shake well to disintegrate, add methanol to make exactly 250 mL, and centrifuge. Pipet a volume of the supernatant, equivalent to about 8 mg of emorfazone ($C_{11}H_{17}N_3O_3$), add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of emorfazone for assay, previously dried in vacuum at 60ºC for 4 hours, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of emorfazone to that of the internal standard.

Amount (mg) of emorfazone ($C_{11}H_{17}N_3O_3$)
$= W_S \times (Q_T/Q_S) \times (2/5)$

W_S: Amount (mg) of emorfazone for assay

Internal standard solution—A solution of 2,4-dinitrophenylhidrazine in methanol (3 in 2000). Prepare before use.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 313 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 25ºC.
Mobile phase: A mixture of water and methanol (11:10)
Flow rate: Adjust the flow rate so that the retention time of emorfazone 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, emorfazone 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of emorfazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ephedrine Hydrochloride Tablets

エフェドリン塩酸塩錠

Add the following next to the Uniformity of dosage units:

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ephedrine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Ephedrine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ephedrine hydrochloride for assay, previously dried at 105ºC for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas , A_T and A_S, of ephedrine in each solution.

Dissolution rate (%) with respect to the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO{\cdot}HCl$)
$= W_S \times (A_T/A_S) \times (1/C) \times 90$

W_S: Amount (mg) of ephedrine hydrochloride for assay
C: Labeled amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO{\cdot}HCl$) in 1 tablet

Operating conditions—
Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.
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 factor of the peak of ephedrine are not less than 10000 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 ephedrine is not more than 2.0%.

Ergometrine Maleate Injection

エルゴメトリンマレイン酸塩注射液

Add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 1500 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Erythromycin Enteric-Coated Tablets

エリスロマイシン腸溶錠

Change the Disintegration to read:

Disintegration <*6.09*> It meets the requirement. For the test with 2nd fluid for disintegration test, use the disk.

Estradiol Benzoate Injection (Aqueous Suspension)

エストラジオール安息香酸エステル水性懸濁注射液

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: no readily detectable foreign insoluble matters are observed.

Sterility <*4.06*> Perform the test according to the Direct method: it meets the requirement.

Estriol Injection (Aqueous Suspension)

エストリオール水性懸濁注射液

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: no readily detectable foreign insoluble matters are observed.

Sterility <*4.06*> Perform the test according to the Direct method: it meets the requirement.

Ethenzamide

エテンザミド

Change the following to read:

Description Ethenzamide occurs as white crystals or crystalline powder.

It is soluble in methanol, in ethanol (95), and in acetone, and practically insoluble in water.

It begins to sublime slightly at about 105ºC.

Identification (1) Determine the absorption spectrum of a solution of Ethenzamide in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethenzamide 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 Ethenzamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ethenzamide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Ethinylestradiol

エチニルエストラジオール

Change the following to read:

Optical rotation <*2.49*> $[\alpha]_D^{20}$: –26 - –31º (after drying, 0.1 g, pyridine, 25 mL, 100 mm).

Famotidine Powder

ファモチジン散

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Famotidine Powder in single-unit containers meets the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Famotidine Powder, add 10 mL of water per 10 mg of famotidine ($C_8H_{15}N_7O_2S_3$), shake well, add 10 mL of methanol, shake well, add methanol to make exactly *V* mL so that each mL contains about 0.4 mg of famotidine ($C_8H_{15}N_7O_2S_3$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$)
$= W_S \times (Q_T/Q_S) \times (V/250)$

W_S: Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Faropenem Sodium for Syrup

シロップ用ファロペネムナトリウム

Add the following next to the Uniformity of dosage unit:

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Faropenem Sodium for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Faropenem Sodium for Syrup, equivalent to about 50 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Faropenem Sodium Reference Standard, equivalent to about 18 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 306 nm.

Dissolution rate (%) with respect to the labeled amount of faropenem ($C_{12}H_{15}NO_5S$)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 225$

W_S: Amount [mg (potency)] of Faropenem Sodium Reference Standard
W_T: Amount (g) of Faropenem Sodium for Syrup
C: Labeled amount [mg (potency)] of faropenem ($C_{12}H_{15}NO_5S$) in 1 g

Faropenem Sodium Tablets

ファロペネムナトリウム錠

Delete the Disintegration and add the following next to the Uniformity of dosage unit:

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Faropenem Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Faropenem Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg (potency) of Faropenem Sodium Hydrate according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Faropenem Sodium Reference Standard, equivalent to about 18 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 306 nm.

Dissolution rate (%) with respect to the labeled amount of faropenem ($C_{12}H_{15}NO_5S$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 225$

W_S: Amount [mg (potency)] of Faropenem Sodium Reference Standard
C: Labeled amount [mg (potency)] of faropenem ($C_{12}H_{15}NO_5S$) in 1 tablet

Flopropione

フロプロピオン

Change the Origin/limits of content, Description, Identification and Purity to read:

Flopropione contains not less than 98.0% and not more than 101.0% of $C_9H_{10}O_4$, calculated on the anhydrous basis.

Description Flopropione occurs as a white to pale yellow-brown crystalline powder.

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

Identification (1) Determine the absorption spectrum of a solution of Flopropione in ethanol (99.5) (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Flopropione as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals *<1.07>* —Proceed with 1.0 g of Flopropione 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 Flopropione 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of flopropione obtained from the sample solution is not larger than 1/10 times the peak area of flopropione from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 267 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 35ºC.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (114:86:1)

Flow rate: Adjust the flow rate so that the retention time of flopropione is about 3 minutes.

Time span of measurement: About 7 times as long as the retention time of flopropione.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of flopropione obtained from 20 μL of this solution is equivalent to 7 to 13% of that of flopropione from the standard solution.

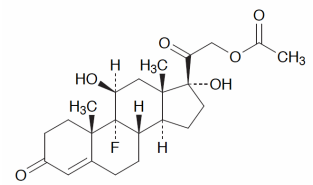
System performance: Dissolve 25 mg of ethyl parahydroxybenzoate in 30 mL of acetonitrile, and add the mobile phase to make 50 mL. To 2.5 mL of this solution add 2 mL of the sample solution and the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, flopropione and ethyl 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 flopropione is not more than 1.0%.

Add the following:

Fludrocortisone Acetate

フルドロコルチゾン酢酸エステル



$C_{23}H_{31}FO_6$: 422.49

9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate [*514-36-3*]

Fludrocortisone Acetate, when dried, contains not less than 97.5% and not more than 102.5% of $C_{23}H_{31}FO_6$.

Description Fludrocortisone Acetate occurs as a white to pale yellow crystals or crystalline powder.

It is soluble in acetone, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 220ºC (with decomposition).

Identification **(1)** Prepare the test solution by proceeding with 10 mg of Fludrocortisone Acetate according to the Oxygen Flask Combustion Method <*1.06*>, using 0.5 mL of 0.01 mol/L sodium hydroxide VS as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> for fluoride.

(2) Determine the absorption spectrum of a solution of Fludrocortisone Acetate in ethanol (95) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fludrocortisone Acetate Reference Standard in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fludrocortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Fludrocortisone Acetate Reference Standard, previously dried: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +131 - +138º (after drying, 0.1 g, acetone, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Fludrocortisone Acetate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Fludrocortisone Acetate in 10 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 50 mL, and use this solution as the stan-

dard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of fludrocortisone acetate obtained from the sample solution is not larger than 1/4 times the peak area of fludrocortisone acetate from the standard solution, and the total area of the peaks other than the peak of fludrocortisone acetate obtained from the sample solution is not larger than 1/2 times the peak area of fludrocortisone acetate 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 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: A mixture of water and tetrahydrofuran (13:7)

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

Time span of measurement: About 2 times as long as the retention time of fludrocortisone acetate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of fludrocortisone acetate obtained from 20 μL of this solution is equivalent to 4.0 to 6.0% of that of fludrocortisone acetate from the standard solution.

System performance: Dissolve 2 mg each of Fludrocortisone Acetate and hydrocortisone acetate in 50 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrocortisone acetate and fludrocortisone acetate 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fludrocortisone acetate is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, 100ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fludrocortisone Acetate and Fludrocortisone Acetate Reference Standard, previously dried, and dissolve separately in ethanol (95) to make exactly 100 mL. Pipet 4 mL each of these solutions, add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 238 nm.

Amount (mg) of fludrocortisone acetate ($C_{23}H_{31}FO_6$) $= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Fludrocortisone Acetate Reference Standard

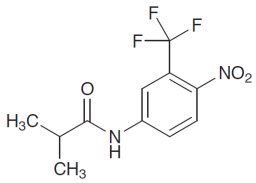
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Flutamide

フルタミド



$C_{11}H_{11}F_3N_2O_3$: 276.21

2-Methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide

[*13311-84-7*]

Flutamide, when dried, contains not less than 98.5% and not more than 101.5% of $C_{11}H_{11}F_3N_2O_3$.

Description Flutamide occurs as a light yellow 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 Flutamide in ethanol (95) (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Flutamide 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 Flutamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Flutamide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 109 - 113ºC

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Flutamide 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 40 mg of Flutamide in 50 mL of methanol, and use this solution as the sample solution. Perform the test with exactly 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the

amounts of them by the area percentage method: the amount of each peak other than flutamide is not more than 0.3%, and the total amount of the peaks other than flutamide is not more than 0.5%.

Operating conditions—

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

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

Time span of measurement: About 2 times as long as the retention time of flutamide, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 1 mL of the standard solution, add methanol to make exactly 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 methanol to make exactly 20 mL. Confirm that the peak area of flutamide obtained from 10 μL of this solution is equivalent to 7 to 13% of that of flutamide from the solution for system suitability test.

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 flutamide is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, in vacuum , phosphorus (V) oxide, 60ºC, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 40 mg each of Flutamide and Flutamide Reference Standard, and dissolve separately in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of flutamide to that of the internal standard, respectively.

$$\text{Amount (mg) of flutamide } (C_{11}H_{11}F_3N_2O_3) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Flutamide Reference Standard

Internal standard solution—A solution of testosterone in methanol (9 in 10000)

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 25ºC.

Mobile phase: A mixture of methanol and 0.05 mol/L potassium dihydrogen phosphate (7:4)

Flow rate: Adjust the flow rate so that the retention time of flutamide 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, flutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

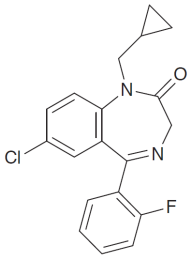
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flutamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Flutoprazepam

フルトプラゼパム



$C_{19}H_{16}ClFN_2O$: 342.79

7-Chloro-1-cyclopropylmethyl-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one [*25967-29-7*]

Flutoprazepam, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{16}ClFN_2O$.

Description Flutoprazepam occurs as a white to light yellow crystals or crystalline powder.

It is freely soluble in ethyl acetate, soluble in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Flutoprazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Flutoprazepam as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Flutoprazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 118 - 122ºC

Purity **(1)** Chloride <*1.03*>—To 1.0 g of Flutoprazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. Pipet 20 mL of the filtrate, 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 with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Flutoprazepam 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).

(3) Related substances—Dissolve 0.10 g of Flutoprazepam in 20 mL of ethyl acetate, and use this solution as the sample solution. Pipet 1 mL of this solution, and add ethyl acetate to make exactly 50 mL. Pipet 1 mL of this solution, add ethyl acetate to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.20% (1 g, 105ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Flutoprazepam, previously dried, dissolve in 70 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.28 mg of $C_{19}H_{16}ClFN_2O$

Containers and storage Containers—Well-closed containers.

Add the following:

Flutoprazepam Tablets

フルトプラゼパム錠

Flutoprazepam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of flutoprazepam ($C_{19}H_{16}ClFN_2O$: 342.79).

Method of preparation Prepare as directed under Tablets, with Flutoprazepam.

Identification To a quantity of powdered Flutoprazepam Tablets, equivalent to 10 mg of Flutoprazepam according to the labeled amount, add 20 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, and add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 240 nm and 244 nm, between 279 nm and 285 nm, and between 369 nm and 375 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Flutoprazepam Tablets add 60 mL of the mobile phase, shake for 15 minutes to disintegrate, disperse the particle with the aid of ultrasonic waves, and add the mobile phase to make exactly V mL so that each mL contains about 20 μg of flutoprazepam ($C_{19}H_{16}ClFN_2O$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of flutoprazepam ($C_{19}H_{16}ClFN_2O$)
$= W_S \times (A_T/A_S) \times (V/1000)$

W_S: Amount (mg) of flutoprazepam for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Flutoprazepam Tablets is not less than 70%.

Start the test with 1 tablet of Flutoprazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.2 μg of flutoprazepam ($C_{19}H_{16}ClFN_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of flutoprazepam for assay, previously dried at 105ºC for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add water 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of flutoprazepam, A_T and A_S, of both solution.

Dissolution rate (%) with respect to the labeled amount of flutoprazepam ($C_{19}H_{16}ClFN_2O$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 9$

W_S: Amount (mg) of flutoprazepam for assay
C: Labeled amount (mg) of flutoprazepam ($C_{19}H_{16}ClFN_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

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 factor of the peak of flutoprazepam are not less than 4000 and not more than 1.5, 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 flutoprazepam is not more than 1.0%.

Assay Weigh accurately not less than 20 Flutoprazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of flutoprazepam ($C_{19}H_{16}ClFN_2O$), add 60 mL of the mobile phase, shake for 15 minutes, and add the mobile phase to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of flutoprazepam for assay, previously dried at 105ºC for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas , A_T and A_S, of flutoprazepam in each solution.

$$\text{Amount (mg) of flutoprazepam } (C_{19}H_{16}ClFN_2O) = W_S \times (A_T/A_S) \times (1/10)$$

W_S: Amount (mg) of flutoprazepam for assay

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 40ºC.

Mobile phase: A mixture of methanol and water (3:1)

Flow rate: Adjust the flow rate so that the retention time of flutoprazepam 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 number of theoretical plates and the symmetry factor of the peak of flutoprazepam are not less than 4000 and not more than 1.5, 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 flutoprazepam is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fosfomycin Sodium for Injection

注射用ホスホマイシンナトリウム

Change the Identification (2) and Water to read:

Identification (2) To 2 mL of a solution of Fosfomycin Sodium for Injection (1 in 250) add 1 mL of perchloric acid and 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphtol-4-sulfonic acid TS, and allow to stand for 30 minutes: a blue color develops.

Water *<2.48>* Not more than 4.0% (0.1 g, coulometric titration).

Add the following:

Furosemide Injection

フロセミド注射液

Furosemide 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 furosemide ($C_{12}H_{11}ClN_2O_5S$: 330.74).

Method of preparation Prepare as directed under Injection, with Furosemide.

Description Furosemide Injection is a colorless, clear liquid.

Identification (1) To a volume of Furosemide Injection, equivalent to 2.5 mg of Furosemide according to the labeled amount, add 10 mL of 2 mol/L hydrochloric acid TS, heat under a reflux condenser on a water bath for 15 minutes. After cooling, render the solution slightly acid with 18 mL of sodium hydroxide TS: the solution responds to the Qualitative Tests *<1.09>* for primary aromatic amines. The color of the solution is red to red-purple.

(2) To a volume of Furosemide Injection, equivalent to 20 mg of Furosemide according to the labeled amount, add water to make 100 mL. Pipet 2 mL of this solution, add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nm, and between 330 nm and 336 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Pipet a volume of Furosemide Injection, equivalent to 40 mg of Furosemide according to the labeled amount, add 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge this solution, to 1.0 mL of the supernatant liquid

add 3.0 mL of water, cool in a ice bath, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. To this solution add 1.0 mL of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of *N*,*N*-diethyl-*N*′-1-naphtylethylenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Determine the absorbance of this solution at 530 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution, prepared in the same manner with 1.0 mL of acetone, as the blank: the absorbance is not more than 0.10.

Bacterial endotoxins <*4.01*> Less than 1.25 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exactly measured volume of Furosemide Injection, equivalent to about 20 mg of Furosemide ($C_{12}H_{11}ClN_2O_5S$), add water to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydrochloride TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide Reference Standard, previously dried at 105ºC for 4 hours, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.01 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 271 nm.

$$\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) = W_S \times (A_T/A_S)$$

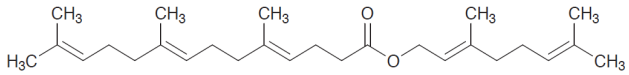
W_S: Amount (mg) of Furosemide Reference Standard

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Add the following:

Gefarnate

ゲファルナート



$C_{27}H_{44}O_2$: 400.64
(2*E*)-3,7-Dimethylocta-2,6-dienyl(4*E*,8*E*)-5,9,13-trimethyltetradeca-4,8,12-trienoate
[*51-77-4*, 4*E* isomer]

Gefarnate is a mixture of 4*E* geometrical isomer.
It contains not less than 98.0% and not more than 101.0% of $C_{27}H_{44}O_2$.

Description Gefarnate is a light yellow to yellow, clear oily liquid.

It is miscible with acetonitrile, with ethanol (99.5) and with cyclohexane.

It is practically insoluble in water.

Identification Determine the infrared absorption spectrum of Gefarnate as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Gefarnate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <*2.56*> d_{20}^{20}: 0.906 - 0.914

Purity **(1)** Acidity—To 1.0 g of Gefarnate add 30 mL of neutralized ethanol. To this solution add 1 drop of phenolphthalein TS and 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Gefarnate 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) Related substances—Use a solution of Gefarnate in acetonitrile (1 in 500) as the sample solution. Pipet 2 mL of this 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of gefarnate obtained from the sample solution is not larger than 1/2 times the peak area of gefarnate from the standard solution, and the total area of the peaks other than the peak of gefarnate obtained from the sample solution is not larger than the peak area of gefarnate 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 gefarnate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of gefarnate obtained from 2 μL of this solution is equivalent to 7 to 13% of that of gefarnate from the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of gefarnate are not less than 4000, and between 0.9 and 1.2, respectively.

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 peak area of gefarnate is not more than 1.0%.

Isomer ratio To 1 mL of Gefarnate add 100 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with 4 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention time of about 37 minutes, where Aa is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_a/(A_a + A_b)$ is between 0.2 and 0.3.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 160 cm in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 5% on acid-treated and silanized siliceous earth for gas chromatography (149 to 177 μm in particle diameter).

Column temperature: A constant temperature of about 210ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the reaction time of the peak showing earlier elution of the two peaks of gefarnate is about 35 minutes.

System suitability—

System performance: When the procedure is run with 4 μL of the sample solution under the above conditions: the resolution between the two peaks of gefarnate is not less than 1.0.

System repeatability: When the test is repeated 6 times with 4 μL of the sample solution under the above operating conditions: the relative standard deviation of the peak area of gefarnate with the shorter retention time of the two peaks is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Gefarnate and Gefarnate Reference Standard, add exactly 5 mL of the internal standard solution and 20 mL of acetonitrile, and use these solutions as the sample solution and standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of gefarnate to that of the internal standard.

$$\text{Amount (mg) of gefarnate } (C_{27}H_{44}O_2) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Gefarnate Reference Standard

Internal standard solution—A solution of tris (4-*t*-butylphenyl) phosphate in acetonitrile (1 in 400)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 40ºC.

Mobile phase: A mixture of acetonitrile for liquid chromatography, water and phosphoric acid (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of gefarnate is about 19 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and gefarnate 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 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gefarnate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and under nitrogen atmosphere.

Add the following:

Gentamicin Sulfate Ophthalmic Solution

ゲンタマイシン硫酸塩点眼液

Gentamicin Sulfate Ophthalmic Solution is an aqueous ophthalmic solution

It contains not less than 90.0% and not more than 110.0% of the labeled potency of expressed as mass of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Method of preparation Prepare as directed under Ophthalmic Solution, with Gentamicin Sulfate.

Description Gentamicin Sulfate Ophthalmic Solution is a clear, colorless or pale yellow liquid.

Identification To a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to 10 mg (potency) of Gentamicin Sulfate according to the labeled amount, add water to make 5 mL, and use this solution as the sample solution. Separately, dissolve an amount of Gentamicin Sulfate Reference Standard, equivalent to 10 mg (potency), in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mixture of chloroform, ammonia solution

(28) and methanol (2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat the plate at 100ºC for 5 minutes: 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.

pH *<2.54>* 5.5 - 7.5

Foreign insoluble matter *<6.11>* It meets the requirement.

Insoluble particulate matter *<6.08>* It meets the requirement.

Sterility *<4.06>* Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics *<4.02>* according to the following conditions.

(i) Test organism, agar media for seed and base layer, agar medium for transferring test organism, and standard solutions—Proceed as directed in the Assay under Gentamicin Sulfate.

(ii) Sample solutions—Pipet a volume of Gentamicin Sulfate Ophthalmic Solution, equivalent to about 12 mg (potency) of Gentamicin Sulfate, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make a solution so that each mL contains about 1 mg (potency). Pipet a suitable volume 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 low concentration sample solution, respectively.

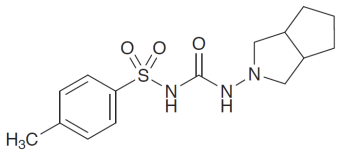
Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Add the following:

Gliclazide

グリクラジド



$C_{15}H_{21}N_3O_3S$: 323.41

1-(Hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulfonyl]urea [*21187-98-4*]

Gliclazide, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{21}N_3O_3S$.

Description Gliclazide is a white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Gliclazide in methanol (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Gliclazide as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point *<2.60>* 165 - 169ºC.

Purity **(1)** Heavy metals *<1.07>*—Proceed with 2.0 g of Gliclazide 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—Conduct this procedure within 2 hours after preparation of the sample solution. Dissolve 50 mg of Gliclazide in 23 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, and add a mixture of water and acetonitrile (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and acetonitrile (11:9) 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than gliclazide obtained from the sample solution is not larger than the peak area of gliclazide from the standard solution, and the total area of the peaks other than the peak of gliclazide obtained from the sample solution is not larger than 3 times the peak area of gliclazide from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.9 with respect to gliclazide, after multiplying by their relative response factor, 5.65.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 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 (4 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: A mixture of water, acetonitrile, triethylamine and trifluoroacetic acid (550:450:1:1)

Flow rate: Adjust the flow rate so that the retention time of gliclazide is about 14 minutes.

Time span of measurement: About 2 times as long as the retention time of gliclazide, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add a mixture of water and acetonitrile (11:9) to make exactly 20 mL. Confirm that the peak area of gliclazide obtained from 20 μL of this solution is equivalent to 10 to 30% of that of gliclazide from 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 factor of the peak of gliclazide are not less than 8000 and not more than 1.5, 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 gliclazide is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, 105ºC, 2 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Gliclazide, previously dried, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.34 mg of $C_{15}H_{21}N_3O_3S$

Containers and storage Containers—Well-closed containers.

Griseofulvin Tablets

グリセオフルビン錠

Delete the Disintegration and add the following next to the Uniformity of dosage units:

Dissolution *<6.10>* When the test is performed at 100 revolutions per minute according to the Paddle method using 900 mL of a solution of sodium laurylsulfate (1 in 100) as the dissolution medium, the dissolution rate in 120 minutes of Griseofulvin Tablets is not less than 70%.

Start the test with 1 tablet of Griseofulvin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 6.9 μg (potency) of Griseofulvin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Griseofulvin Reference Standard, equivalent to about 28 mg (potency), and dissolve in ethanol (95) to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of the dissolution medium and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 295 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank

Dissolution rate (%) with respect to the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (45/2)$

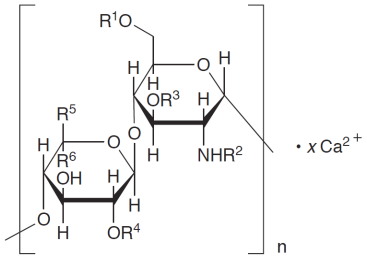
W_S: Amount [mg (potency)] of Griseofulvin Reference Standard

C: Labeled amount [mg (potency)] of griseofulvin ($C_{17}H_{17}ClO_6$) in 1 tablet

Add the following:

Heparin Calcium

ヘパリンカルシウム



R^1, R^3, R^4 = SO_3^- or H
R^2 = SO_3^- or 
R^5 = CO_2^-, R^6 = H
or
R^5 = H, R^6 = CO_2^-

[*37270-89-6*]

Heparin Calcium is the calcium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

Heparin Calcium prolongs the clotting time of blood. It contains not less than 150 Heparin Units per mg.

Heparin Calcium, calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units, and not less than 8.0% and not more than 12.0% of calcium (Ca: 40.08).

Description Heparin Calcium occurs as a white to grayish brown powder or grains. It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 10 mg of Heparin Calcium in 5 mL of water. To this solution add 0.1 mL of 1 mol/L hydrochloric acid TS and 5 mL of a solution of toluidine blue O (1 in 20000): a purple to red-purple color develops.

(2) Dissolve 50 mg of Heparin Calcium in 5 mL of water: the solution responds to the Qualitative Tests *<1.09>* for calcium salt.

pH *<2.54>* Dissolve 1.0 g of Heparin Calcium in 100 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Heparin Calcium in 20 mL of water: the solution is clear. De-

termine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.05.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of Heparin Calcium. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals <*1.07*>—Proceed with 0.5 g of Heparin Calcium according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(4) Barium—Dissolve 30 mg of Heparin Calcium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(5) Residual solvent—Being specified separately.

(6) Total nitrogen—Weigh accurately about 0.1 g of Heparin Calcium, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(7) Protein—To 1.0 mL of the sample solution obtained in (4) add 5 drops of a solution of trichloroacetic acid (1 in 5): neither a precipitate nor turbidity is produced.

(8) Oversulfated chondroitin sulfate—Dissolve 20 mg of Heparin Calcium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10000), and use this solution as the sample solution. Determine the spectrum of the sample solution, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H) in accordance with the following conditions, using spectrometer (1) with a proton resonance frequency of not less than 400 MHz: no signal occurs from the *N*-acetyl proton of oversulfated chondroitin sulfate between δ 2.13 ppm and δ 2.23 ppm.

Operating conditions—

Temperature: 25ºC

Spinning: Off

Number of data points: 32,768

Spectral range: ±6.0 ppm with the DHO signal at the center

Flip angle: 90º

Delay time: 20 seconds

Number of dummy scans: 4

Number of scans: Repeat until the S/N ratio of the signal of *N*-acetyl proton of heparin is not less than 200.

Window function: Exponential function (Line broadening factor = 0.2 Hz)

System suitability—

Dissolve 0.10 mg of Oversulfated Chondroitin Sulfate Reference Standard in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10000), and use this solution as the standard solution. Dissolve about 20 mg of heparin calcium in 0.60 mL of the standard solution, and use this solution as the solution for system suitability test. Proceed under the above operating conditions: Signals occur from the *N*-acetyl protons of heparin and oversulfated chondroitin sulfate at δ 2.02 – 2.06 ppm and at δ 2.13 – δ 2.23 ppm, respectively.

Loss on drying <*2.41*> Not more than 8% (50 mg, in vacuum, 60ºC, 3 hours).

Bacterial endotoxins <*4.01*> Less than 0.0030 EU/heparin Unit.

Assay (1) Heparin

(i) Substrate solution: Dissolve 15 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ-OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 20 mL of water.

(ii) Activated blood coagulation factor X solution: Dissolve bovine activated blood coagulation factor X in water to make a solution containing 0.426 Unit per mL.

(iii) Buffer solution: Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 750 mL of water, adjust the pH to 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(iv) Reaction stop solution: To 20 mL of acetic acid (100) add water to make 40 mL.

(v) Heparin standard solutions: Dissolve Heparin Sodium Reference Standard in isotonic sodium chloride solution to make a solution containing 10 Units per mL. To 100 μL of this solution add buffer solution to make exactly 5 mL, and use this solution as the standard stock solution. Prepare the heparin standard solutions (1), (2), (3), (4) and (5) by addition of antithrombin III TS, human normal plasma and the buffer solution to the standard solution as directed in the following table.

Heparin standard solution		Buffer solution (μL)	Antithrombin III TS (μL)	Human normal plasma (μL)	Standard solution (μL)
No.	Heparin concentration (Unit/mL)				
(1)	0	800	100	100	0
(2)	0.02	700	100	100	100
(3)	0.04	600	100	100	200
(4)	0.06	500	100	100	300
(5)	0.08	400	100	100	400

(vi) Sample solution: Weigh accurately an adequate amount of Heparin Calcium, dissolve in isotonic sodium chloride solution so that each mL contains about 0.5 Units according to the labeled amount. To 100 μL of this solution add 100 μL of antithrombin III TS, 100 μL of human normal plasma and 700 μL of the buffer solution, and use this solution as the sample solution.

(vii) Procedure: Transfer 400 μL of the sample solution to a test tube, and warm at 37ºC for 4 minutes. To this solution add 200 μL of the activated blood coagulation factor X solution, mix well, warm at 37ºC for exactly 30 seconds, add 400 μL of the substrate solution, previously warmed at 37ºC, and mix well. Warm this solution at 37ºC for exactly 3 minutes, add 600 μL of the reaction stop solution, and mix immediately. Determine the absorbance at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the blank solution prepared by addition of 600 μL of the reaction stop solution and 600 μL of water to 400 μL of the sample solution. Proceed the same way with the heparin standard solution (1), the heparin standard solution (2), the heparin standard solution (3), the heparin standard solution (4) and the heparin standard solution (5), and determine their absorbances.

(viii) Calculation: Plot the absorbances of the standard solutions and their heparin concentrations to prepare a calibration curve. Determine the heparin concentration, *C*, of the sample solution, and calculate heparin Units per mg of Heparin Calcium from the following formula.

Units per mg of Heparin Calcium = $C \times 10 \times (b/a)$

a: Amount of sample (mg)

b: Total volume (mL) of isotonic sodium chloride solution used to dissolve the sample to make the solution containing about 0.5 Units per mL

(2) Calcium Weigh accurately about 50 mg of Heparin Calcium, dissolve in 20 mL of water, add 2 mL of 8 mol/L sodium hydroxide TS, allow to stand for 3 to 5 minutes with occasional shaking, add 0.1 g of NN indicator, and immediately titrate <*2.50*> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

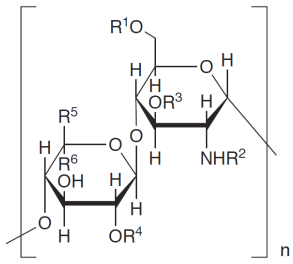
Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.4008 mg of Ca

Containers and storage Containers—Tight containers.

Heparin Sodium

ヘパリンナトリウム

Add the following next to the Japanese title:



R^1, R^3, R^4 = SO_3Na or H

R^2 = SO_3Na or 

R^5 = CO_2Na, R^6 = H

or

R^5 = H, R^6 = CO_2Na

[*9041-08-1*]

Change the Origin/limits of content to read:

Heparin Sodium is the sodium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals. It prolongs the clotting time of blood. Heparin Sodium obtained from the livers and the lungs contains not less than 110 Heparin Units per mg, and that obtained from the intestinal mucosa contains not less than 130 Heparin Units per mg.

Heparin Sodium, calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units.

Label the name of the organ used as the starting material.

Human Menopausal Gonadotrophin

ヒト下垂体性性腺刺激ホルモン

Change the Purity to read:

Purity Interstitial cell-stimulating hormone—Perform the test according to the following method: the ratio of the unit of interstitial cell-stimulating hormone (luteinizing hormone) to that of follicle-stimulating hormone is not more than 1. The luteinizing activity of the hormone is determined by the seminal vesicle weight assay or ovarian ascorbic acid depletion assay. The seminal vesicle weight assay may be used when the ratio of the unit of interstitial cell-stimulating hormone to that of follicle-stimulating hormone is not more than 1 and not less than 0.10.

1. Seminal vesicle weight assay

(i) Test animals—Select healthy male albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin Reference Standard, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare three kinds of solutions, containing 10, 20 and 40 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their seminal vesicles as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the seminal vesicle 20 to 35 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, and designate this solution as the low-dose standard solution, S_L. Store these solutions at 2 - 8ºC.

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose sample solution, T_H and the low-dose sample solution, T_L, so that their concentrations are similar to those of the corresponding standard solutions, respectively. Store these solutions at 2 - 8ºC.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously once every day 0.2 mL each of S_H, S_L, T_H and T_L to each animal in the respective groups for five days. On the sixth day, excise the seminal vesicles, remove extraneous tissue, remove fluid adhering to the vesicles and the contents of the vesicles by lightly pressing between filter papers, and weigh the vesicles.

(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the mass of seminal vesicles to read.

2. Ovarian ascorbic acid depletion assay

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Dissolve Human Menopausal Gonadotrophin Reference Standard in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare four kinds of solutions, containing 2, 4, 8 and 16 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and determine the amount of ovarian ascorbic acid. Separately, inject bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to a control group of animals. According to the result of the test, designate the concentration of the reference standard, which will make the amount of ovarian ascorbic acid 0.80 to 0.85 times that in the control group, as the concentration for the low-dose standard solution, and 4 to 6 times that as the concentration for the high-dose standard solution. Dissolve Human Menopausal Gonadotrophin Reference Standard in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose standard solution and low-dose standard solution to contain the concentrations described above, and designate them as S_H and S_L, respectively.

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose sample solution and low-dose sample solution to contain units equal to those of the high-dose standard solution and low-dose standard solution, and designate them as T_H and T_L, respectively.

(iv) Procedure—Inject subcutaneously to each animal 80 units of serum gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. At 56 to 72 hours after the injection, inject subcutaneously to each animal 40 units of human chorionic gonadotrophin dissolved in 0.5 mL of isotonic sodium chloride solution. On 6 to 9 days after the last injection, divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject 1 mL each of S_H, S_L, T_H and T_L into the tail vein of each animal in groups A, B, C and D, respectively. At 2 to 4 hours after the injection, excise the both ovaries, remove the fat and other unwanted tissues attached to the ovaries, weigh, add a prescribed volume between 5 and 15 mL of metaphosphoric acid solution (1 in 40), homogenize with a homogenizer on ice, and centrifuge. To 0.5 to 1 mL (1 mL in principle. 0.5 mL may be used when the absorbance is not more than 0.1) of the supernatant liquid, add 1.5 mL of metaphosphoric acid solution (1 in 40) and 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, and immediately determine the absorbance of the solution at 520 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Separately, weigh accurately 10.0 mg of Ascorbic Acid Reference Standard, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet a suitable volume of this solution, and add metaphosphoric acid solution (1 in 40) to make a solution so that each mL contains 2.0 to 10.0 μg of ascorbic acid ($C_6H_8O_6$: 176.12). To 2.5 mL of this solution, add 2.5 mL of 2,6-dichloroindophenol sodium-sodium acetate TS, mix the solution, immediately determine the absorbance in the same manner as mentioned above, and prepare the calibration curve. From the calibration curve of ascorbic acid, determine the amount (mg) of ascorbic acid in 100 g of ovary.

(v) Calculation—Proceed as directed in (v) in the Assay by changing the mass of ovaries to the amount of ascorbic acid to read.

Hydralazine Hydrochloride for Injection

注射用ヒドララジン塩酸塩

Add the following next to the pH:

Bacterial endotoxins <*4.01*> Less than 5.0 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test. (T: 106.0%)

Foreign insoluble matter <*6.06*> Perform the test according

to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Hydrocortisone Sodium Phosphate

ヒドロコルチゾンリン酸エステルナトリウム

Delete the Loss on drying and change the Origin/limits of content, Optical rotation and Purity (5) to read:

Hydrocortisone Sodium Phosphate contains not less than 96.0% and not more than 102.0% of $C_{21}H_{29}Na_2O_8P$, calculated on the anhydrous basis.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +123 - +131º (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

Purity (5) Free phosphoric acid—Weigh accurately about 0.25 g of Hydrocortisone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution into separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1ºC for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, at 740 nm of the sample solution and Standard Phosphoric Acid Solution: the amount of free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = (A_T/A_S) \times (1/W) \times 258.0$$

W: Amount (mg) of Hydrocortisone Sodium Phosphate, calculated on the anhydrous basis.

Add the following next to the Purity:

Water <*2.48*> Not more than 5.0% (30 mg. coulometric titration).

Change the Assay to read:

Assay Weigh accurately about 20 mg each of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard (previously determine the water content <*2.48*> in the same manner as Hydrocortisone Sodium Phosphate), 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 standard solution as directed under Liquid Chromatography <*2.01*> 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.

$$\text{Amount (mg) of hydrocortisone sodium phosphate } (C_{21}H_{29}Na_2O_8P) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Hydrocortisone Sodium Phosphate Reference Standard, calculated on the anhydrous 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 25ºC.

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 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 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%.

Hydroxypropylcellulose

ヒドロキシプロピルセルロース

Add the following next to the Japanese title:

[*9004-64-2*]

Low Substitute Hydroxypropylcellulose

低置換度ヒドロキシプロピルセルロース

Add the following next to the Japanese title:

[*9004-64-2*, Hydroxypropylcellulose]

Hypromellose

ヒプロメロース

Change the Chemical name to read:

[*9004-65-3*]

Hypromellose Phthalate

ヒプロメロースフタル酸エステル

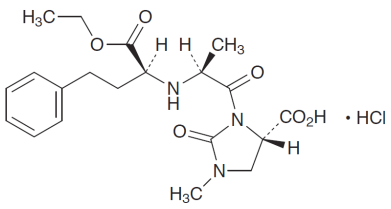
Change the Chemical name to read:

[*9050-31-1*]

Add the following:

Imidapril Hydrochloride

イミダプリル塩酸塩



$C_{20}H_{27}N_3O_6$·HCl: 441.91
(4*S*)-3-{(2*S*)-2-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropylamino]propanoyl}-1-methyl-2-oxoimidazolidine-4-carboxylic acid monohydrochloride
[*89396-94-1*]

Imidapril Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{20}H_{27}N_3O_6$·HCl.

Description Imidapril Hydrochloride occurs as a white crystals.
It is freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).
Dissolve 1.0 g of Imidapril Hydrochloride in 100 mL of water: the pH of the solution is about 2.
Melting point: about 203ºC (with decomposition).

Identification **(1)** To 3 mL of a solution of Imidapril Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Imidapril Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Imidapril Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: –65.0 - –69.0º (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Imidapril Hydrochloride 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—Dissolve 25 mg of Imidapril Hydrochloride in 50 mL of the mobile phase, and use this solution as the test 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 with respect to imidapril, obtained from the sample solution, is not larger than 2/5 times the peak area of imidapril from the standard solution, and the area of each peak other than the peaks of imidapril and other than those mentioned above from the sample solution is not larger than 1/5 times the peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than imidapril from the sample solution is not larger than 1/2 times the peak area of imidapril 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 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40ºC.
Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.
Flow rate: Adjust the flow rate so that the retention time of imidapril is about 8 minutes.
Time span of measurement: About 2 times as long as the retention time of imidapril, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of imidapril obtained from 20 μL of this solution is equivalent to 7 to 13% of that of imidapril from 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 factor of the peak of imidapril are not less than 5000 and not more than 1.5, 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 imidapril is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 3

hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Imidapril Hydrochloride, previously dried, dissolve in 70 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS from the first equivalent point to the second equivalent point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 44.19 mg of $C_{20}H_{27}N_3O_6$·HCl

Containers and storage Containers—Well-closed containers.

Add the following:

Imidapril Hydrochloride Tablets

イミダプリル塩酸塩錠

Imidapril Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Imidapril Hydrochloride ($C_{20}H_{27}N_3O_6$·HCl: 441.91).

Method of preparation Prepare as directed under Tablets, with Imidapril Hydrochloride.

Identification Weigh accurately an amount of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride, add 5 mL of ethanol (99.5), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 25 mg of imidapril hydrochloride in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, ethyl acetate, water, ethanol (99.5) and acetic acid (100) (16:16:7:2:2) to a distance of about 13 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.

Purity Related substances— To a quantity of powdered Imidapril Hydrochloride Tablets, equivalent to 25 mg of Imidapril Hydrochloride according to the labeled amount, add 40 mL of diluted methanol (2 in 5), shake vigorously for 10 minutes, add diluted ethanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of this solution, add diluted methanol (2 in 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.45 with respect to imidapril, obtained from the sample solution, is not larger than the peak area of imidapril from the standard solution; the area of the peak having the relative retention time of about 0.8 with respect to imidapril from the sample solution is not larger than 7/10 times the peak area of imidapril from the standard solution; and the area of each peak other than the peak of imidapril and other than those mentioned above from the sample solution is not larger than 3/10 times the peak area of imidapril from the standard solution. Furthermore, the total area of the peaks other than the peak of imidapril from the sample solution is not larger than 1.5 times the peak area of imidapril 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 imidapril, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add diluted methanol (2 in 5) to make exactly 20 mL. Confirm that the peak area of imidapril obtained from 20 μL of this solution is equivalent to 7 to 13% of that of imidapril from 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 factor of the peak of imidapril are not less than 5000 and not more than 1.5, 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 imidapril is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Imidapril Hydrochloride Tablets add $2V/5$ mL of water, shake vigorously for 10 minutes, add diluted methanol (2 in 3) to make exactly V mL so that each mL contains about 0.1 mg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl), filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of imidapril for assay, previously dried at 105ºC for 3 hours, dissolve in diluted methanol (2 in 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas , A_T and A_S, of imidapril in each solution.

Amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl)
$= W_S \times (A_T/A_S) \times (V/100)$

W_S: Amount (mg) of imidapril hydrochloride for assay

Operating conditions—

Proceed as directed in the operating conditions in the Assay
System suitability—

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 factor of the peak of imidapril are not less than 5000 and not more than 1.5, 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 imidapril is not more than 1.0%.

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Imidapril Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Imidapril Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of imidapril hydrochloride for assay, previously dried at 105ºC for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of imidapril in each solution.

Dissolution rate (%) with respect to the labeled amount of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 9$

W_S: Amount (mg) of imidapril hydrochloride for assay
C: Labeled amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.
System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of imidapril are not less than 5000 and not more than 1.5, respectively.

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 imidapril is not more than 2.0%.

Assay Weigh accurately not less than 20 Imidapril Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl), add 30 mL of diluted methanol (2 in 5) and exactly 5 mL of the internal standard solution, shake vigorously for 10 minutes, add diluted methanol (2 in 5) to make 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of imidapril hydrochloride for assay, previously dried at 105ºC for 3 hours, dissolve in 5 mL of the internal standard solution, add diluted methanol (2 in 5) to make 50 mL. Pipet 5 mL of this solution, add diluted methanol (2 in 5) to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of imidapril to that of the internal standard.

Amount (mg) of imidapril hydrochloride ($C_{20}H_{27}N_3O_6$·HCl)
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of imidapril hydrochloride for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (2 in 5) (1 in 500)
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 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 40ºC.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.7 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of imidapril 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, imidapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

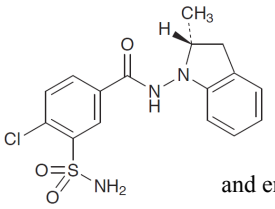
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of imidapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Indapamide

インダパミド



and enantiomer

$C_{16}H_{16}ClN_3O_3S$: 365.83

4-Chloro-*N*-[(2*RS*)-2-methyl-2,3-dihydro-1*H*-indol-1-yl]-3-sulfamoylbenzamide [*26807-65-8*]

Indapamide contains not less than 98.5% and not more than 101.5% of $C_{16}H_{16}ClN_3O_3S$, calculated on the dried basis.

Description Indapamide occurs as a white crystalline powder.

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

A solution of Indapamide in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Indapamide in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indapamide 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 Indapamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Indapamide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Indapamide as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 167 - 171ºC.

Purity **(1)** Chloride <*1.03*>—To 1.5 g of Indapamide add 50 mL of water, shake for 15 minutes, allow to stand in an ice bath for 30 minutes, and filter. To 30 mL of the filtrate 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 with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.01%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Indapamide 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) Related substances— Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Indapamide in 5 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution, standard solution (1) and standard solution (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100) (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution (1), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1) and (2), is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 110ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Indapamide and Indapamide Reference Standard (separately, determine the loss on drying <*2.41*> in the same manner as Indapamide), and dissolve each in a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of indapamide to that of the internal standard.

$$\text{Amount (mg) of indapamide } (C_{16}H_{16}ClN_3O_3S) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Indapamide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 287 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 40ºC.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000), acetonitrile and methanol (6:3:1).

Flow rate: Adjust the flow rate so that the retention time of

indapamide 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, indapamide 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 peak area of indapamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Indapamide Tablets

インダパミド錠

Indapamide Tablets contain not less than 93.0% and not more than 103.0% of the labeled amount of indapamide ($C_{16}H_{16}ClN_3O_3S$: 365.83).

Method of preparation Prepare as directed under Tablets, with Indapamide.

Identification To an amount of powdered Indapamide Tablets, equivalent to 10 mg of Indapamide according to the labeled amount, add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Indapamide Reference Standard in 5 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, cyclohexane and acetic acid (100) (100:80:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show a blue-purple color and have the same R_f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Indapamide Tablets add exactly V/10 mL of the internal standard solution, and add a mixture of water and ethanol (99.5) (1:1) to make V mL so that each mL contains about 0.1 mg of indapamide ($C_{16}H_{16}ClN_3O_3S$), shake to disintegrate, treat with ultrasonic waves for 10 minutes, shake again for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of indapamide ($C_{16}H_{16}ClN_3O_3S$)
$= W_S \times (Q_T/Q_S) \times (V/200)$

W_S: Amount (mg) of Indapamide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rates in 45 minutes of 1-mg tablets and in 90 minutes of 2-mg tablets are not less than 70%, respectively.

Start the test with 1 tablet of Indapamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 1.1 μg of indapamide ($C_{16}H_{16}ClN_3O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Indapamide Reference Standard (separately, determine the loss on drying <*2.41*> in the same manner as Indapamide), and dissolve in ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas , A_T and A_S, of indapamide in each solution.

Dissolution rate (%) with respect to the labeled amount of indapamide ($C_{16}H_{16}ClN_3O_3S$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/2)$

W_S: Amount (mg) of Indapamide Reference Standard, calculated on the dried basis

C: Labeled amount (mg) of indapamide ($C_{16}H_{16}ClN_3O_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Indapamide.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of indapamide are not less than 3500 and not more than 1.5, respectively.

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 indapamide is not more than 1.5%.

Assay To 20 Indapamide Tablets add 80 mL of a mixture of water and ethanol (99.5) (1:1), shake well to disintegrate, and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add a mixture of water and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet a volume of indapamide

($C_{16}H_{16}ClN_3O_3S$), equivalent to about 2 mg, and add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make exactly 20 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Indapamide Reference Standard (separately, determine the loss on drying <*2.41*> in the same manner as Indapamide), and dissolve in a mixture of water and ethanol (99.5) (1:1) to make 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution and a mixture of water and ethanol (99.5) (1:1) to make 20 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Indapamide.

$$\text{Amount (mg) of indapamide } (C_{16}H_{16}ClN_3O_3S) = W_S \times (Q_T/Q_S) \times (1/10)$$

W_S: Amount [mg (potency)] of Indapamide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in a mixture of water and ethanol (99.5) (1:1) (3 in 1000)

Containers and storage Containers—Tight containers.

Indigocarmine Injection

インジゴカルミン注射液

Add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 7.5 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> Perform the test according to Method 2: it meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Indometacin Suppositories

インドメタシン坐剤

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Indometacin Suppositories add 80 mL of a mixture of methanol and acetic acid (100) (200:1), dissolve by warming, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet V mL of this solution, equivalent to about 2 mg of indometacin ($C_{19}H_{16}ClNO_4$), add a mixture of methanol and acetic acid (100) (200:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Indometacin Reference Standard, previously dried at 105ºC for 4 hours, and dissolve in a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and acetic acid (100) (200:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 320 nm.

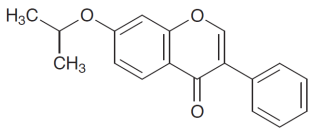
$$\text{Amount (mg) of indometacin } (C_{19}H_{16}ClNO_4) = W_S \times (A_T/A_S) \times (2/V)$$

W_S: Amount (mg) of Indometacin Reference Standard

Add the following:

Ipriflavone

イプリフラボン



$C_{18}H_{16}O_3$: 280.32
7-(1-Methylethyl)oxy-3-phenyl-4*H*-chromen-4-one
[*35212-22-7*]

Ipriflavone, when dried, contains not less than 98.5% and not more than 101.0% of $C_{18}H_{16}O_3$.

Description Ipriflavone occurs as white to yellowish white crystals or crystalline powder.

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

It gradually turns yellow on exposure to light.

Identification (1) Determine the absorption spectrum of a solution of Ipriflavone in methanol (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ipriflavone 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 Ipriflavone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ipriflavone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 116 - 119ºC.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of

Ipriflavone 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 <*1.11*>—Prepare the test solution with 1.0 g of Ipriflavone according to Method 4, and perform the test. Prepare the test solution with 10 mL of dilute hydrochloric acid instead of using 3 mL of hydrochloric acid. Prepare the standard color with 1.0 mL of Standard Arsenic Solution (not more than 1 ppm).

(3) Related substances—Dissolve 30 mg of Ipriflavone in 50 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add acetonitrile 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of ipriflavone obtained from the sample solution is not larger than 1/2 times the peak area of ipriflavone from the standard solution, and the total area of the peaks other than the peak of ipriflavone obtained from the sample solution is not larger than the peak area of ipriflavone from the standard solution.

Operating conditions—

Detector, column, column temperature 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 ipriflavone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of ipriflavone obtained from 20 μL of this solution is equivalent to 7 to 13% of that of ipriflavone from 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 factor of the peak of ipriflavone are not less than 2000 and not more than 1.5, 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 ratio of the peak area of ipriflavone to that of the internal standard is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Ipriflavone and Ipriflavone Reference Standard, previously dried, dissolve separately in acetonitrile to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ipriflavone to that of the internal standard.

$$\text{Amount (mg) of ipriflavone } (C_{18}H_{16}O_3) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Ipriflavone Reference Standard

Internal standard solution—A solution of di-*n*-butyl phthalate in acetonitrile (1 in 100)

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 25ºC.

Mobile phase: A mixture of acetonitrile and water (3:2)

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

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ipriflavone 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 ratio of the peak area of ipriflavone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Ipriflavone Tablets

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Ipriflavone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ipriflavone ($C_{18}H_{16}O_3$: 280.32).

Method of preparation Prepare as directed under Tablets, with Ipriflavone.

Identification To a quantity of powdered Ipriflavone Tablets, equivalent to 11 mg of Ipriflavone according to the labeled amount, add 100 mL of methanol, shake vigorously for 10 minutes, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 247 nm and 251 nm, and between 297 nm and 301 nm.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Ipriflavone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of ipriflavone ($C_{18}H_{16}O_3$), add 30 mL of acetonitrile, shake vigorously for 15 minutes, add acetonitrile to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution. add acetonitrile to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Ipriflavone Reference Standard, previously dried at 105ºC for 2 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Ipriflavone.

$$\text{Amount (mg) of ipriflavone } (C_{18}H_{16}O_3) = W_S \times (Q_T/Q_S)$$

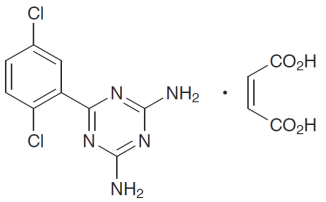
W_S: Amount (mg) of Ipriflavone Reference Standard
Internal standard solution—A solution of di-*n*-butyl phthalate in acetonitrile (1 in 100)

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Irsogladine Maleate

イルソグラジンマレイン酸塩



$C_9H_7Cl_2N_5 \cdot C_4H_4O_4$: 372.16
6-(2,5-Dichlorophenyl)-1,3,5-triazine-2,4-diamine monomaleate [*84504-69-8*]

Irsogladine Maleate, when dried, contains not less than 99.0% and not more than 101.0% of $C_9H_7Cl_2N_5 \cdot C_4H_4O_4$.

Description Irsogladine Maleate occurs as white crystals or crystalline powder. It has a slightly bitter taste.

It is sparingly soluble in acetic acid (100) and in ethyleneglycol, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 20 mg of Irsogladine Maleate in methanol to make 20 mL. Take 2 mL of this solution, and add water to make 20 mL. To 2 mL of this solution add water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Irsogladine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg of Irsogladine Maleate in 1 mL of dilute hydrochloric acid and 4 mL of water, and add 3 drops of potassium permanganate TS: the color of the solution is discharged immediately.

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Irsogladine Maleate 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—Dissolve 50 mg of Irsogladine Maleate in 10 mL of ethyleneglycol, and use this solution as the sample solution. Pipet 1 mL of this solution, add ethyleneglycol 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of maleic acid and irsogladine obtained from the sample solution is not larger than 1/10 times the peak area of irsogladine 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 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: A mixture of methanesulfonic acid solution (1 in 1000) and methanol (4:1)

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

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

System suitability—Test for required detectability: Pipet 1 mL of the standard solution, and add ethyleneglycol to make exactly 10 mL. Confirm that the peak area of irsogladine obtained from 5 μL of this solution is equivalent to 7 to 13% of that of irsogladine from the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of irsogladine are not less than 3000 and not more 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 peak area of irsogladine is

not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, 105ºC, 4 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Irsogladine Maleate, previously dried, dissolve in 25 mL of acetic acid (100), add 25 mL of acetic anhydride, and titrate *<2.50>* with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.61 mg of $C_9H_7Cl_2N_5 \cdot C_4H_4O_4$

Containers and storage Containers—Well-closed containers.

Add the following:

Irsogladine Maleate Fine Granules

イルソグラジンマレイン酸塩細粒

Irsogladine Maleate Fine Granules contain not less than 93.0% and not more than 107.0% of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$: 372.16).

Method of preparation Prepare fine granules as directed under Powders, with Irsogladine Maleate.

Identification To a quantity of powdered Irsogladine Maleate Fine Granules, equivalent to 2 mg of Irsogladine Maleate according to the labeled amount, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same R_f value as the spot from the standard solution.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: Irsogladine Maleate Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

Take out the total contents of 1 pack of Irsogladine Maleate Fine Granules, add 2 mL of water, add 2 mL methanol per mg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$), treat with ultrasonic waves for 10 minutes with occasional shaking, and add water to make exactly V mL so that each mL contains about 40 μg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$). Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add water to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V/500)$

W_S: Amount (mg) of irsogladine maleate for assay

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Irsogladine Maleate Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of Irsogladine Maleate Fine Granules, equivalent to about 4 mg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Dissolution rate (%) with respect to the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 9$

W_S: Amount (mg) of irsogladine maleate for assay
W_T: Amount (g) of Irsogladine Maleate Fine Granules
C: Labeled amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$) in 1 g

Particle size *<6.03>* It meets the requirement.

Assay Weigh accurately an amount of powdered Irsogladine Maleate Fine Granules, equivalent to about 5 mg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$), add exactly 5 mL of the internal standard solution, shake until it is dispersed, and add 5 mL of water. To the solution add 25 mL of ethyleneglycol, treat with ultrasonic waves for 10 minutes with occasional shaking, and add ethyleneglycol to make 50 mL. Filter this solution through a

membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, and dissolve in ethyleneglycol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 5 mL of water and ethyleneglycol to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of irsogladine to that of the internal standard.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= W_S \times (Q_T/Q_S) \times (1/5)$

W_S: Amount (mg) of irsogladine maleate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 2500)

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 25ºC.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (750:250:3)

Flow rate: Adjust the flow rate so that the retention time of irsogladine 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, irsogladine 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 ratio of the peak area of irsogladine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Irsogladine Maleate Tablets

イルソグラジンマレイン酸塩錠

Irsogladine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$: 372.16).

Method of preparation Prepare as directed under Tablets, with Irsogladine Maleate.

Identification To a quantity of powdered Irsogladine Maleate Tablets, equivalent 2 mg of Irsogladine Maleate according to the labeled amount, add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of irsogladine maleate in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of petroleum ether, acetone and acetic acid (100) (12:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same R_f value as the spot from the standard solution.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Irsogladine Maleate Tablets add 2 mL of water, add 2 mL of methanol per mg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$), treat with ultrasonic waves for 10 minutes with occasional shaking, add water to make exactly V mL so that each mL contains about 40 μg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$). Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add water to make exactly 20 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V/500)$

W_S: Amount (mg) of irsogladine maleate for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Irsogladine Maleate Tablets is not less than 80%.

Start the test with 1 tablet of Irsogladine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.2 μg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, and dissolve in

methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add water to make exactly 20 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using water as the blank, and determine the absorbances, A_T and A_S, at 210 nm.

Dissolution rate (%) with respect to the labeled amount of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 9$

W_S: Amount (mg) of irsogladine maleate for assay
C: Labeled amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Irsogladine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$), add exactly 5 mL of the internal standard solution, shake until it is dispersed, and add 5 mL of water. To this solution add 25 mL of ethyleneglycol, treat with ultrasonic waves for 10 minutes with occasional shaking, and add ethyleneglycol to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of irsogladine maleate for assay, previously dried at 105ºC for 4 hours, and dissolve in ethyleneglycol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 5 mL of water and ethyleneglycol to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of irsogladine to that of the internal standard.

Amount (mg) of irsogladine maleate ($C_9H_7Cl_2N_5 \cdot C_4H_4O_4$)
$= W_S \times (Q_T/Q_S) \times (1/5)$

W_S: Amount (mg) of irsogladine maleate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 2500)
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 25ºC.
Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (750:250:3)
Flow rate: Adjust the flow rate so that the retention time of irsogladine 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, irsogladine 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 ratio of the peak area of irsogladine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Isepamicin Sulfate Injection

イセパマイシン硫酸塩注射液

Isepamicin Sulfate Injection is an aqueous injection.
It contains not less than 90.0% and not more than 110.0% of the labeled potency of isepamicin ($C_{22}H_{43}N_5O_{12}$: 569.60).

Method of preparation Prepare as directed under Injections, with Isepamicin Sulfate.

Description Isepamicin Sulfate Injection is a clear, colorless liquid.

Identification To a volume of Isepamicin Sulfate Injection, equivalent to 20 mg (potency) of Isepamicin Sulfate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve an amount of Isepamicin Sulfate Reference Standard, equivalent to 20 mg (potency) in 10 mL of water, and use this solution as the standard solution. Proceed with these solutions as directed in the Identification (2) under Isepamicin Sulfate.

Osmotic pressure ratio Being specified separately.

pH *<2.54>* 5.5 - 7.5

Purity Related substances—Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of the sample solution by the automatic integration method and calculate the amounts of these peaks by the area percentage method: the amount of isoserine, having the relative retention time of about 0.3 with respect to isepamicin, is not more than 2.0%, and the amount of gentamicin B, having the relative retention time of about 1.3 with respect to isepamicin, is not more than 4.0%. For this calculation, use the peak area of gentamicin B after multiplying by the relative response factor, 1.11.
Operating conditions—
Apparatus, detector, column, column temperature, reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reaction reagent: Proceed as directed in the operating conditions in the Assay under Isepamicin Sulfate.
Time span of measurement: About 2 times as long as the re-

tention time of isepamicin.

System suitability—

System performance and system repeatability: Proceed as directed in the Assay under Isepamicin Sulfate.

Test for required detectability: To 1 mL of the sample solution add water to make 10 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 isepamicin obtained from 5 μL of this solution is equivalent to 7 to 13% of that of isepamicin from the solution for system suitability test.

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mg (potency).

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Isepamicin Sulfate Injection, equivalent to about 0.2 g (potency) of Isepamicin Sulfate, add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Isepamicin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Isepamicin Sulfate.

$$\text{Amount [mg (potency)] of isepamicin } (C_{22}H_{43}N_5O_{12}) = W_S \times (A_T/A_S) \times 10$$

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

Containers and storage Containers—Hermetic containers.

Expiration date 24 months after preparation.

Isoniazid Injection

イソニアジド注射液

Add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Isoniazid Tablets

イソニアジド錠

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isoniazid Tablets add exactly V mL of water so that each mL, contains about 0.5 mg of isoniazid ($C_6H_7N_3O$), and shake well to disintegrate. Filter this solution, 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. Proceed as directed in the Assay.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = W_S \times (A_T/A_S) \times (V/100)$$

W_S: Amount (mg) of isoniazid for assay

Isosorbide Dinitrate Tablets

硝酸イソソルビド錠

Change the Origin/limits of content to read:

Isosorbide Dinitrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$: 236.14).

Add the following next to the Purity:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Isosorbide Dinitrate Tablets add 1 mL of water, and shake to disintegrate. To this solution add a mixture of water and methanol (1:1) to make exactly V mL so that each mL contains about 0.1 mg of isosorbide dinitrate ($C_6H_8N_2O_8$), and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of isosorbide dinitrate } (C_6H_8N_2O_8) = W_S \times (A_T/A_S) \times V \times (1/500)$$

W_S: Amount (mg) of isosorbide dinitrate for assay, calculated on the anhydrous basis

Change the Assay to read:

Assay Weigh accurately the mass of not less than 20 tablets of Isosorbide Dinitrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of isosorbide dinitrate tablets ($C_6H_8N_2O_8$), add a mixture of water and methanol (1:1) to make exactly 50 mL, and shake for 10 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of isosorbide dinitrate for assay (separately, determine the water content <*2.48*> in the same manner as Isosorbide Dinitrate), dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of water and methanol (1:1) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of isosorbide dinitrate in each solution.

$$\text{Amount (mg) of isosorbide dinitrate } (C_6H_8N_2O_8) = W_S \times (A_T/A_S) \times (1/10)$$

W_S: Amount (mg) of isosorbide dinitrate for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 40ºC.

Mobile phase: A mixture of water and methanol (11:9)

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

System suitability—

System performance: When the procedure is run with10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide dinitrate are not less than 3000 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 isosorbide dinitrate is not more than 1.0%.

Josamycin Tablets

ジョサマイシン錠

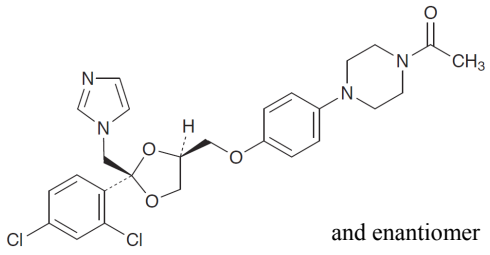
Change the Disintegration to read:

Disintegration <*6.09*> Perform the test using the disk, it meets the requirement.

Add the following:

Ketoconazole

ケトコナゾール



and enantiomer

$C_{26}H_{28}Cl_2N_4O_4$:531.43

1-Acetyl-4-(4-{[(2*RS*,4*SR*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazine [*65277-42-1*]

Ketoconazole, when dried, contains not less than 99.0% and not more than 101.0% of $C_{26}H_{28}Cl_2N_4O_4$.

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

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

A solution of Ketoconazole in methanol (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Ketoconazole in methanol (3 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Ketoconazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Ketoconazole as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 148 - 152ºC.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Ketoconazole 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) Related Substances Dissolve 0.10 g of Ketoconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method:

the area of each peak other than the peak of ketoconazole obtained from the sample solution is not larger than 2/5 times the peak area of ketoconazole from the standard solution, and the total area of the peaks other than the peak of ketoconazole obtained from the sample solution is not larger than the peak area of ketoconazole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 (3 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase A: Acetonitrile for liquid chromatography.

Mobile phase B: A solution of tetrabutylammonium hydrogensulfate (17 in 5000).

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 (vol%)	Mobile phase B (vol%)
0 – 10	5 → 50	95 → 50
10 – 15	50	50

Flow rate: 2.0 mL per minute.

Time span of measurement: Beginning after the solvent peak to 15 minutes after injection.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of ketoconazole obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ketoconazole from 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 factor of the peak of ketoconazole are not less than 40000 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 ketoconazole is not more than 2.5%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ketoconazole, previously dried, dissolve in 70 mL of a mixture of 2-butanone and acetic acid (100) (7:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.57 mg of $C_{26}H_{28}Cl_2N_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Ketoconazole Cream

ケトコナゾールクリーム

Ketoconazole Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$:531.43)

Method of preparation Prepare as directed under Ointments, with Ketoconazole.

Identification To a quantity of Ketoconazole Cream, equivalent to 0.1 g of Ketoconazole according to the labeled amount, add 20 mL of 2-propanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of ketoconazole in 5 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:25:2:1) to a distance of about 12 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.

Assay Weigh accurately an amount of Ketoconazole Cream, equivalent to about 25 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ketoconazole for assay, previously dried at 105ºC for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

Amount (mg) of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$)
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of ketoconazole for assay

Internal standard solution—A solution of xanthone in methanol (1 in 10000)

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 40ºC.

Mobile phase: To ammonium acetate solution (1 in 200) add acetic acid (100) to adjust the pH to 5.0. To 250 mL of this solution add 750 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of ketoconazole 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 ketoconazole 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 ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Ketoconazole Lotion

ケトコナゾールローション

Ketoconazole Lotion is an emulsion lotion.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$:531.43).

Method of preparation Prepare as directed under Lotions, with Ketoconazole.

Description Ketoconazole Lotion occurs as a white emulsion.

Identification Shake well and take an amount of Ketoconazole Lotion, equivalent to 0.1 g of Ketoconazole according to the labeled amount, add 20 mL of 2-propanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of ketoconazole in 5 mL of 2-propanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:25:2:1) to a distance of about 12 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.

Assay Shake well and weigh accurately an amount of Ketoconazole Lotion, equivalent to about 25 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ketoconazole for assay, previously dried at 105ºC for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

$$\text{Amount (mg) of ketoconazole } (C_{26}H_{28}Cl_2N_4O_4) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of ketoconazole for assay

Internal standard solution—A solution of xanthone in methanol (1 in 10000)

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 40ºC.

Mobile phase: To ammonium acetate solution (1 in 200) add acetic acid (100) to adjust the pH to 5.0. To 250 mL of this solution add 750 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of ketoconazole 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 ketoconazole 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 ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Ketoconazole Solution

ケトコナゾール液

Ketoconazole Solution is a liquid for external use.

Ketoconazole Solution contains not less than 95.0% and not more than 105.0% of the labeled amount of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$:531.43).

Method of preparation Prepare as directed under Solutions with Ketoconazole.

Description Ketoconazole Solution is a clear liquid.

Identification To a volume of Ketoconazole Solution, equivalent to 10 mg of Ketoconazole according to the labeled amount, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ketoconazole in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol, water and ammonia solution (28) (40:40:30:2:1) 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.

pH Being specified separately.

Assay Weigh accurately an amount of Ketoconazole Solution, equivalent to about 10 mg of ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$), add exactly 5 mL of the internal standard solution, and add 15 mL of methanol. To 1 mL of this solution add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ketoconazole for assay, previously dried at 105ºC for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 20 mL. Take 1 mL of this solution, add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ketoconazole to that of the internal standard.

$$\text{Amount (mg) of ketoconazole } (C_{26}H_{28}Cl_2N_4O_4) = W_S \times (Q_T/Q_S) \times (1/5)$$

W_S: Amount (mg) of ketoconazole for assay

Internal standard solution—A solution of bifonazole in methanol (3 in 2000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 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 40ºC.

Mobile phase: A mixture of a solution of diisopropylamine in methanol (1 in 500), ammonium acetate solution (1 in 200) and acetic acid (100) (1800:600:1).

Flow rate: Adjust the flow rate so that the retention time of ketoconazole is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ketoconazole 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 ratio of the peak area of ketoconazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖

Change the Purity (2) and Isomer ratio to read:

Purity (2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

Isomer ratio Place 1 mg of Anhydrous Lactose in a 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (18:7), seal the vial tightly with a screw cap, and mix gently. Allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the peak areas of α-lactose derivateive and β-lactose derivateive, A_a and A_b, and calculate the contents (%) of α-lactose derivateive and β-lactose derivateive in Anhydrous Lactose by the following equations.

$$\text{Content (\%) of } \alpha\text{-lactose} = [A_a / (A_a + A_b)] \times 100$$
$$\text{Content (\%) of } \beta\text{-lactose} = [A_b / (A_a + A_b)] \times 100$$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Injection port temperature: A constant temperature of about 275ºC

Detector temperature: A constant temperature of about 275ºC.

Column: A glass column 4 mm in inside diameter and 90 cm in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopro-

pyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215ºC.

Carrier gas: Helium

Flow rate: A constant flow rate of about 40 mL per minute.

System suitability—

System performance: Prepare a solution with 1 mg of a mixture of *α*-lactose and *β*-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 *μ*L of this solution under the above operating conditions, and determine the retention times of the peaks of *α*-lactose derivative and *β*-lactose derivative: the relative retention time of *α*-lactose derivative with respect to that of *β*-lactose derivative is about 0.7 with the resolution between these peaks being not less than 3.0.

Lactose Hydrate

乳糖水和物

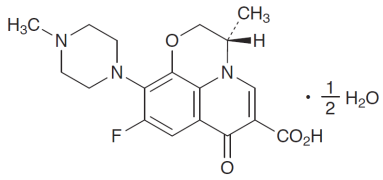
Change the Purity (2) to read:

Purity (2) Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pale red color or red color.

Add the following:

Levofloxacin Hydrate

レボフロキサシン水和物



$C_{18}H_{20}FN_3O_4 \cdot ½H_2O$: 370.38

(3*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate [*138199-71-0*]

Levofloxacin Hydrate contains not less than 99.0% and not more than 101.0% of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37), caluculated on the anhydrous basis.

Description Levofloxacin Hydrate occurs as light yellowish white to yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually turns dark light yellowish white on exposure to light.

Melting point: about 226ºC (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Levofloxacin Hydrate in 0.1 mol/L hydrochloric acid solution (1 in 150000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Levofloxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: –92 - –99º (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Levofloxacin Hydrate 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 using light-resistant vessels. Dissolve 50 mg of Levofloxacin Hydrate in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of this solution, and add a mixture of water and methanol (1:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 *μ*L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 1.2 with respect to levofloxacin obtained from the sample solution is not larger than 2/5 times the peak area of levofloxacin from the standard solution, and the area of each peak other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 1/5 times the peak area of levofloxacin from the standard solution. Furthermore, the total area of the peaks other than the peak of levofloxacin and other than the peak having the relative retention time of about 1.2 with respect to levofloxacin from the sample solution is not larger than 3/10 times the peak area of levofloxacin from the standard solution.

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 *μ*m in particle diameter).

Column temperature: A constant temperature of about 45ºC.

Mobile phase: Dissolve 1.76 g of L-valine, 7.71 g of ammonium acetate and 1.25 g of Copper (II) sulfate pentahydrate in water to make 1000 mL. To this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of levofloxacin is about 22 minutes.

Time span of measurement: About 2 times as long as the retention time of levofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of levofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that of levofloxacin from the standard solution.

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 μL of th is solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 with respect to levofloxacin is 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 levofloxacin is not more than 3.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> 2.1 - 2.7% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Levofloxacin Hydrate, dissolve in 100 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, 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.

Lidocaine Injection

リドカイン注射液

Delete the Pyrogen and add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 1.0 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Lincomycin Hydrochloride Injection

リンコマイシン塩酸塩注射液

Lincomycin Hydrochloride Injection is an aqueous injection.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Method of preparation Prepare as directed under Injections, with Lincomycin Hydrochloride Hydrate.

Description: Lincomycin Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Lincomycin Hydrochloride Injection, equivalent to 30 mg (potency) of Lincomycin Hydrochloride Hydrate, add 30 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Lincomycin Hydrochloride Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Dissolve 150 g of ammonium acetate in 800 mL of water, adjust the pH to 9.6 with ammonia solution (28), and add water to make 1000 mL. To 80 mL of this solution add 40 mL of 2-propanol and 90 mL of ethyl acetate, shake, develop the plate with the upper layer of this solution to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate (1 in 1000) on the plate: the principal spots from the sample solution and the standard solution show the same R_f value.

pH <*2.54*> 3.5 - 5.5

Bacterial endotoxins <*4.01*> Less than 0.50 EU/mg (potency).

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Pipet a volume of Lincomycin Hydrochloride Injection, equivalent to about 0.3 g (potency) of Lincomycin Hydrochloride Hydrate, add the mobile phase to make exactly 30 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lincomycin Hydrochloride Ref-

erence Standard, equivalent to 20 mg (potency), dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Lincomycin Hydrochloride Hydrate.

Amount [mg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$)
$= W_S \times (A_T/A_S) \times 15$

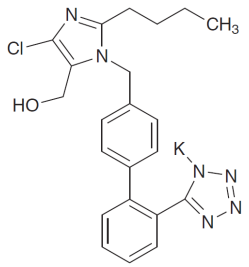
W_S: Amount [mg (potency)] of Lincomycin Hydrochloride Reference Standard

Containers and storage Containers—Hermetic containers.

Add the following:

Losartan Potassium

ロサルタンカリウム



$C_{22}H_{22}ClKN_6O$: 461.00
Monopotassium 5-{[4′-(2-butyl-4-chloro-5-hydroxymethyl-1*H*-imidazol-1-yl)methyl]biphenyl-2-yl}-1*H*-tetrazol-1-ide
[*124750-99-8*]

Losartan Potassium contains not less than 98.5% and not more than 101.0% of $C_{22}H_{22}ClKN_6O$, calculated on the anhydrous basis.

Description Losartan Potassium occurs as a white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Losartan Potassium in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Losartan 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 Losartan Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Losartan Potassium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Losartan Potassium responds to the Qualitative Tests <*1.09*> (1) for potassium salt.

(4) Perform the test with Losartan Potassium as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Losartan Potassium 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 30 mg of Losartan Potassium in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peaks of solvent and losartan obtained from the sample solution is not larger than 1/10 times the peak area of losartan from the standard solution, and the total area of the peaks other than the peak of losartan from the sample solution is not larger than 3/10 times the peak area of losartan from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 25ºC.

Mobile phase A: Diluted phosphoric acid (1 in 1000).

Mobile phase B: Acetonitrile.

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 (vol%)	Mobile phase B (vol%)
0 – 25	75 → 10	25 → 90
25 – 35	10	90

Flow rate: 1.0 mL per minute.

Time span of measurement: 35 minutes after injection of the sample.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of losartan obtained from 10 μL of this solution is equivalent to 7 to 13% of that of losartan from 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 factor of the peak of losartan are not less than 10000 and not more than 1.3, 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 losartan is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 0.5% (0.25 g, volumetric titration, direct titration)

Assay Weigh accurately about 25 mg each of Losartan Potassium and Losartan Potassium Reference Standard (separately, determine the water content <*2.48*> in the same manner as Losartan Potassium), dissolve separately in methanol to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, A_T and A_S, of losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Losartan Potassium Reference Standard, calculated on the anhydrous basis.

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 (5 μm in particle diameter).

Column temperature: A constant temperature of about 35ºC.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2)

Flow rate: Adjust the flow rate so that the retention time of losartan 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 number of theoretical plates and the symmetry factor of the peak of losartan are not less than 5500 and not more than 1.4, respectively.

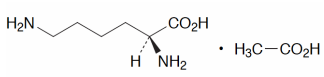
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

L-Lysine Acetate

L - リジン酢酸塩



$C_6H_{14}N_2O_2 \cdot C_2H_4O_2$: 206.24
(2*S*)-2,6-Diaminohexanoic acid monoacetate
[*57282-49-2*]

L-Lysine Acetate, when dried, contains not less than 98.5% and not more than 101.0% of $C_6H_{14}N_2O_2 \cdot C_2H_4O_2$.

Description L-Lysine Acetate occurs as white crystals or crystalline powder. It has a characteristic odor and a slightly acid taste.

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

It is deliquescent.

Identification **(1)** Determine the infrared absorption spectrum of L-Lysine Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Lysine Acetate (1 in 20) responds to the Qualitative Tests <*1.09*> (2) for acetate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +8.5 - +10.0º (after drying, 2.5 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the pH of the solution is between 6.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Lysine Acetate in 10 mL of water: the solution is colorless and clear.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Lysine Acetate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Lysine Acetate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Lysine Acetate. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Lysine Acetate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Lysine Acetate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0

mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Lysine Acetate, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately 2.5 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-valine, L-cystine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 100 mL, Pipet 2 mL of this solution, add 0.02 mol/L hydrochloric acid to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the test solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Based on the peak heights of the amino acids obtained from the sample solution and standard solution, determine the mass of the amino acids other than lysine contained in 1 mL of the sample solution, and calculate the mass per cent: the amount of each amino acids other than lysine is not more than 0.1%.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 570 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57ºC.

Chemical reaction bath temperature: A constant temperature of about 130ºC.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and to each phase add 0.1 mL of capric acid.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	-
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	-
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	-
Sodium hydroxide	-	-	-	-	8.00 g
Ethanol (99.5)	260 mL	20 mL	4 mL	-	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	-	-
Benzyl alcohol	-	-	-	5 mL	-
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount
Total volume	2000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing mobile phases: Proceed with 20 μL of the standard solution under the above operating conditions: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine and arginine are eluted in this order. Switchover the mobile phases A, B, C, D and E in sequence so that the resolution between the peaks of isoleucine and leucine is not less than 1.2.

Reaction reagents: Dissolve 407 g of lithium acetate dihydrate in water, and add 245 mL of acetic acid (100), 801 mL of 1-methoxy-2-propanol, and water to make 2000 mL, gas with nitrogen for 10 minutes, and use this solution as the solution (I). Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin, gas with nitrogen for 5 minutes, add 0.134 g of sodium borohydride, and gas the solution with nitrogen for 30 minutes. To this solution add the solution (I) (12:13).

Mobile phase flow rate: 0.32 mL per minute.

Reaction reagent flow rate: 0.30 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is 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 peak height of each amino acid in the standard solution is not more than 5.0%, and the relative standard deviation of the retention time is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 80ºC, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Acetate, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.31 mg of $C_6H_{14}N_2O_2 \cdot C_2H_4O_2$

Containers and storage Containers—Tight containers.

Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Delete the Bacterial endotoxins and add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Meglumine Sodium Amidotorizoate Injection

アミドトリゾ酸ナトリウムメグルミン注射液

Delete the Pyrogen and change the Method of preparation to read:

Method of preparation

(1)	
Amidotorizoic Acid (anhydrous)	471.78 g
Sodium Hydroxide	5.03 g
Meglumine	125.46 g
Water for Injection	a sufficient quantity
	To make 1000 mL
(2)	
Amidotorizoic Acid (anhydrous)	597.30 g
Sodium Hydroxide	6.29 g
Meglumine	159.24 g
Water for Injection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients (1) or (2).

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Change the Origin/limits of content, Description and Identification to read:

Mepivacaine Hydrochloride 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 mepivacaine hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$: 282.81).

Description Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

Identification To a volume of Mepivacaine Hydrochloride Injection, equivalent to 20 mg of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrometry <*2.24*>: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

Add the following next to the Identification

pH Being specified separately.

Bacterial endotoxins <*4.01*> Less than 0.6 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Change the Assay to read:

Assay Pipet a volume of Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of Mepivacaine Hydrochloride ($C_{15}H_{22}N_2O \cdot HCl$) according to the labeled amount, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105ºC for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloride TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of mepivacaine

to that of the internal standard.

Amount (mg) of mepivacaine hydrochloride ($C_{15}H_{22}N_2O$·HCl)
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of mepivacaine hydrochloride for assay

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).
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 25ºC.
Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and acetonitrile (11:9).
Flow rate: Adjust the flow rate so that the retention time of mepivacaine 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, mepivacaine 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of mepivacaine to that of the internal standard is not more than 1.0%.

Add the following:

Meropenem for Injection

注射用メロペネム

Meropenem for Injection is a preparation for injection, which is dissolved before use.
It contains not less than 93.0% and not more than 107.0% of the labeled potency of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

Method of preparation Prepare as directed under Injections, with Meropenem Hydrate.

Description Meropenem for Injection occurs as a white to light yellow crystalline powder.

Identification Determine the infrared absorption spectrum of Meropenem for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 3410 cm^{-1}, 1750 cm^{-1}, 1655 cm^{-1}, 1583 cm^{-1} and 1391 cm^{-1}.

pH <*2.54*> Dissolve an amount of Meropenem for Injection, equivalent to 0.25 g (potency) of Meropenem Hydrate according to the labeled amount, in 5 mL of water: the pH of the solution is between 7.3 and 8.3.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Meropenem for Injection, equivalent to 1.0 g (potency) of Meropenem Hydrate according to the labeled amount, in 20 mL of water: the solution is clear and is not more intensely colored than the following matching fluid.
Matching fluid: To a mixture of 0.3 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 1.2 mL of Iron (III) Chloride Colorimetric Stock Solution add 18.5 mL of diluted hydrochloric acid (1 in 40).
(2) Related substances—Being specified separately.

Loss on drying <*2.41*> 9.5 - 12.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60ºC, 3 hours).

Bacterial endotoxins <*4.01*> Less than 0.12 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass on the contents of not less than 10 containers of Meropenem for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Meropenem Hydrate, dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Meropenem Reference Standard, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of meropenem to that of the internal standard.

Amount [mg (potency)] of meropenem ($C_{17}H_{25}N_3O_5S$)
$= W_S \times (Q_T/Q_S)$

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

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300)
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Meropenem Hydrate.

System suitability—

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

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 meropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Methylcellulose

メチルセルロース

Change the Chemical name to read:

[*9004-67-5*]

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Change the Assay to read:

Assay Weigh accurately the mass of the contents of not less than 10 containers of Minocycline Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mobile phase to make exactly 100 mL. Pipet 25 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area of minocycline, A_T and A_S, of each solution.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
$= W_S \times (A_T/A_S) \times 4$

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

Operating conditions—

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay under Minocycline Hydrochloride.

Mobile phase: Adjust the pH to 6.5 of a mixture of ammonium oxalate monohydrate solution (7 in 250), *N*,*N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) with tetrabutylammonium hydroxide TS.

System suitability—

System performance: Dissolve 50 mg of minocycline hydrochloride in water to make 25 mL. Heat 5 mL of this solution on a water bath for 60 minutes, and add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline 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 minocycline is not more than 1.0%.

Add the following:

Minocycline Hydrochloride Tablets

ミノサイクリン塩酸塩錠

Minocycline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of Minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation Prepare as directed under Tablets, with Minocycline Hydrochloride.

Identification To a quantity of powdered Minocycline Hydrochloride Tablets, equivalent to 10 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 625 mL of a solution of hydrochloric acid in methanol (19 in 20000), shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 221 nm and 225 nm, between 261 nm and 265 nm, and between 354 nm and 358 nm.

Purity Related substances—Conduct this procedure rapidly after preparation of the sample solution. Powder not less than 5 Minocycline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to 50 mg (potency) of Minocycline Hydrochloride according to the labeled amount, add 60 mL of the mobile phase, shake vigorously, and add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area from both solutions by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the amount of the peak of epiminocycline, having the relative retention time of 0.83 with respect to minocycline, is not more than 2.0%.

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 2.5 times as long as the retention time of minocycline, beginning after the solvent peak.

System suitability—

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

Test for required detectability: To 2 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 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 from 20 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 20μL 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%.

Water <*2.48*> Not more than 12.0% (0.5 g of powdered Minocycline Hydrochloride Tablets, volumetric titration, back titration)

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Minocycline Hydrochloride Tablets add 60 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg (potency) of Minocycline Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
$= W_S \times (A_T/A_S) \times (V/50)$

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

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Minocycline Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Minocycline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 9 μg (potency) of Minocycline Hydrochloride according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride Reference Standard, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 348 nm.

Dissolution rate (%) with respect to the labeled amount of minocycline ($C_{23}H_{27}N_3O_7$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

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

C: Labeled amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$) in 1 tablet

Assay To a number of Minocycline Hydrochloride Tablets, equivalent to about 1 g (potency) of Minocycline Hydrochloride, add 120 mL of the mobile phase, treat with ultrasonic waves for 15 minutes, and add the mobile phase to make exactly 200 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Minocycline Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of minocycline in each solution.

Amount [mg (potency)] of minocycline ($C_{23}H_{27}N_3O_7$)
$= W_S \times (A_T/A_S) \times 40$

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

Operating conditions—

Detector, column, column temperature and flow rate: Proceed as directed in the operating conditions in the Assay under Minocycline Hydrochloride.

Mobile phase: Adjust the pH to 6.5 of a mixture of ammonium oxalate monohydrate solution (7 in 250), *N*,*N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) with tetrabutylammonium hydroxide TS.

System suitability—

System performance: Dissolve 50 mg of minocycline hydrochloride in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, and add water to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, epiminocycline and minocycline 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 minocycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 1.5 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

Add the following next to the Uniformity of dosage units:

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Morphine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Morphine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of morphine hydrochloride for assay (separately, determine the water content <*2.48*> in the same manner as Morphine Hydrochloride Hydrate), and dissolve in water to make exactly 100 mL. Pipet 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 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas , A_T and A_S, of morphine in each solution.

Dissolution rate (%) with respect to the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$·HCl·3H_2O)
$= W_S \times (A_T/A_S) \times (1/C) \times 36 \times 1.168$

W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3$·HCl·3H_2O) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

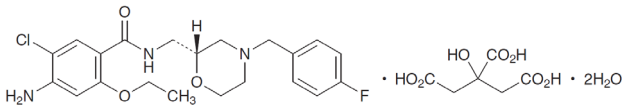
System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of morphine are not less than 5000 and not more than 2.0, respectively.

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 morphine is not more than 2.0%.

Add the following:

Mosapride Citrate Hydrate

モサプリドクエン酸塩水和物



and enantiomer

$C_{21}H_{25}ClFN_3O_3$·$C_6H_8O_7$·2H_2O: 650.05
4-Amino-5-chloro-2-ethoxy-*N*-{[(2*RS*)-4-(4-fluorobenzyl)morpholin-2-yl]methyl}benzamide monocitrate dihydrate [*636582-62-2*]

Mosapride Citrate Hydrate contains not less than 98.5% and not more than 101.0% of mosapride citrate ($C_{21}H_{25}ClFN_3O_3$·$C_6H_8O_7$: 614.02), calculated on the anhydrous basis.

Description Mosapride Citrate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Mosapride Citrate Hydrate in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mosapride Citrate Hydrate in methanol (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Mosapride Citrate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mosapride Citrate Hydrate in *N*,*N*-dimethylformamide (1 in 10) responds to the Qualitative Tests <*1.09*> (1) for citrate.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Mosapride Citrate Hydrate in a platinum crucible 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 0.10 g of Mosapride Citrate Hydrate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.47 with respect to mosapride from the sample solution is not larger than 3 times the peak area of mosapride from the standard solution, and the area of each peak other than the peak of mosapride and other than the peak mentioned above from the sample solution is not larger than the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than the peak of mosapride from the sample solution is not larger than 5 times the peak area of mosapride from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 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 40ºC.

Mobile phase A: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 4.0 with dilute hydrochloric acid, and add water to make 1000 mL.

Mobile phase B: Acetonitrile

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 (vol%)	Mobile phase B (vol%)
0 – 35	80 → 45	20 → 55

Flow rate: 1.0 mL per minute

Time span of measurement: Beginning after the solvent peak to 35 minutes after injection.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of mosapride obtained from 5 μL of this solution is equivalent to 15 to 25% of that of mosapride from the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 40000 and not more 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 peak area of mosapride is not more than 5.0%.

(3) Residual solvent—Being specified separately.

Water *<2.48>* 5.0 - 6.5% (0.5 g, volumetric titration, back titration).

Residue on ignition *<2.44>* Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately 0.5 g of Mosapride Citrate Hydrate, dissolve in 70 mL of acetic acid (100), and titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 61.40 mg of $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Add the following:

Mosapride Citrate Tablets

モサプリドクエン酸塩錠

Mosapride Citrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$: 614.02).

Method of preparation Prepare as directed under Tablets, with Mosapride Citrate Hydrate.

Identification **(1)** To an amount of powdered Mosapride Citrate Tablets, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$), add 10 mL of dilute acetic acid, shake for 10 minutes, and filter. To 5 mL of the filtrate add 0.3 mL of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits maxima between 271 nm and 275 nm, and between 306 nm and 310 nm.

Purity Related substances—Powder not less than 20 tablets of Mosapride Citrate Tablets. Moisten a portion of the powder, equivalent to 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) according to the labeled amount, with 1 mL of water. Add 9 mL of methanol, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks having the relative retention times of about 0.60 and about 0.85 with respect to mosapride from the sample solution is not larger than the peak area of mosapride from the

standard solution, and the area of each peak other than the peak of mosapride and other than those mentioned above from the sample solution is not larger than 2/5 times the peak area of mosapride from the standard solution. Furthermore, the total area of the peaks other than mosapride from the sample solution is not larger than 2 times the peak area of mosapride from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Mosapride Citrate Hydrate.

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 (vol%)	Mobile phase B (vol%)
0 – 40	85 → 45	15 → 55

Time span of measurement: Beginning after the solvent peak to 40 minutes after injection.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 25 mL. Confirm that the peak area of mosapride obtained from 10 μL of this solution is equivalent to 3.0 to 5.0% of that of mosapride from 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 factor of the peak of mosapride are not less than 40000 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 mosapride is not more than 3.0%.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mosapride Citrate Tablets add 5 mL of water, and shake well to disintegrate. Add 20 mL of methanol, shake for 20 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 20 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/50)$

W_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mosapride Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Mosapride Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 2.8 μg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of mosapride citrate for assay (separately, determine the water content *<2.48>* in the same manner as Mosapride Citrate Hydrate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 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 exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas , A_T and A_S, of mosapride in each solution.

Dissolution rate (%) with respect to the labeled amount of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 9$

W_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 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 40ºC.

Mobile phase: Dissolve 8.82 g of trisodium citrate dihydrate in 800 mL of water, adjust the pH to 3.3 with dilute hydrochloric acid, and add water to make 1000 mL. To 240 mL of this solution add 90 mL of methanol and 70 mL of acetonitrile

Flow rate: Adjust the flow rate so that the retention time of mosapride is about 9 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mosapride are not less than 4000 and not more than 2.0, respectively.

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 mosapride is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Mosapride Citrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$), and moisten with 2 mL of water.

Add 70 mL of methanol, shake for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of mosapride citrate for assay (separately, determine the water content <*2.48*> in the same manner as Mosapride Citrate Hydrate), and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 273 nm.

Amount (mg) of mosapride citrate ($C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$) $= W_S \times (A_T/A_S) \times (1/5)$

W_S: Amount (mg) of mosapride citrate for assay, calculated on the anhydrous basis.

Containers and storage Containers—Tight containers.

Nicomol Tablets

ニコモール錠

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Norethisterone

ノルエチステロン

Change the following to read:

Description Norethisterone occurs as a white to pale yellowish white crystalline powder. it has no odor.

It is sparingly soluble in ethanol (95), in acetone, and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

It is affected by light.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: –32 - –37º (after drying, 0.25 g, acetone, 25 mL, 100 mm).

Phenobarbital

フェノバルビタール

Change the Origin/limits of content and Identification to read:

Phenobarbital, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$.

Identification (1) Determine the absorption spectrum of a solution of Phenobarbital in boric acid-potassium chloride-sodium hydrochloride buffer solution, pH 9.6 (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Phenobarbital as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

10% Phenobarbital Powder

フェノバルビタール散 10%

Change the Identification to read:

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 238 nm and 242 nm.

(2) To 6 g of 10% Phenobarbital Powder add 150 mL of ethanol, shake well, and filter. Condense the filtrate in a water bath to about 5 mL, filter with about 50 mL of water to collect the crystals, and dry them at 105ºC for 2 hours. Determine the infrared absorption spectrum of the crystals as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, 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 the Identification:

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 10% Phenobarbital Powder is not less than 80%.

Start the test with an accurately weighted about 0.3 g of 10% Phenobarbital Powder, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this

solution as the sample solution. Separately, weigh accurately about 17 mg of phenobarbital for assay, previously dried at 105ºC for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using a mixture of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6, and water (2:1) as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Dissolution rate (%) with respect to the labeled amount of phenobarbital ($C_{12}H_{12}N_2O_3$)
$= (W_S / W_T) \times (A_T/A_S) \times (1/C) \times 180$

W_S: Amount (mg) of phenobarbital for assay
W_T: Amount (g) of 10% Phenobarbital Powder
C: Labeled amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$) in 1 g

Particle size *<6.03>* It meets the requirement.

Change the Assay to read:

Assay Weigh accurately about 0.2 g of 10% Phenobarbital Powder, dissolve in a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of phenobarbital for assay, previously dried at 105ºC for 2 hours, and add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL. Pipet 5 mL of this solution, add a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using a boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 as the blank, and determine the absorbances, A_T and A_S, at 240 nm.

Amount (mg) of phenobarbital ($C_{12}H_{12}N_2O_3$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of phenobarbital for assay

Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Add the following next to the pH:

Bacterial endotoxins *<4.01>* Less than 7.5 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter *<6.06>* Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter *<6.07>* Perform the test according to Method 2: it meets the requirement.

Sterility *<4.06>* Perform the test according to the Membrane filtration method: it meets the requirement.

Phenytoin Powder

フェニトイン散

Change the Origin/limits of content and Assay to read:

Phenytoin Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Assay Weigh accurately an amount of Phenytoin Powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of methanol, treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105ºC for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard.

Amount (mg) of phenytoin ($C_{15}H_{12}N_2O_2$)
$= W_S \times (Q_T/Q_S) \times 2$

W_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25000)
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 258 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 40ºC.
Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution, pH 3.5 (11:9)
Flow rate: Adjust the flow rate so that the retention time of

phenytoin 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, phenytoin 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 ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Phenytoin Tablets

フェニトイン錠

Change the Origin/limits of content and Identification to read:

Phenytoin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Identification Weigh a portion of powdered Phenytoin Tablets, equivalent to about 0.3 g of Phenytoin, transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL potions of diethyl ether. Combine the extracts, evaporate the diethyl ether in a water bath, and dry the residue at 105ºC for 2 hours. Proceed with the residue as directed in the Identification under Phenytoin.

Add the following next to the Identification:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Phenytoin Tablets add $3V$/5 mL of a mixture of water and acetonitrile (1:1), treat with ultrasonic waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly V mL so that each mL contains about 1 mg of phenytoin ($C_{15}H_{12}N_2O_2$). Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = W_S \times (Q_T/Q_S) \times (V/25)$$

W_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25000)

Change the Assay to read:

Assay Weigh accurately the mass of not less than 20 Phenytoin Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 50 mg of phenytoin ($C_{15}H_{12}N_2O_2$), add 30 mL of a mixture of water and acetonitrile (1:1), treat with ultrasound waves for 15 minutes with occasional shaking, shake for another 10 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of phenytoin for assay, previously dried at 105ºC for 2 hours, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of phenytoin to that of the internal standard.

$$\text{Amount (mg) of phenytoin } (C_{15}H_{12}N_2O_2) = W_S \times (Q_T/Q_S) \times 2$$

W_S: Amount (mg) of phenytoin for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 25000)
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 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 40ºC.

Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution, pH 3.5 (11:9)

Flow rate: Adjust the flow rate so that the retention time of phenytoin is about 5 minutes.
System suitability—

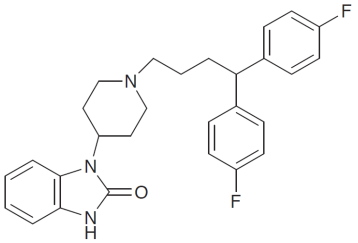
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, phenytoin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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 ratio of the peak area of phenytoin to that of the internal standard is not more than 1.0%.

Add the following

Pimozide

ピモジド



$C_{28}H_{29}F_2N_3O$: 461.55

1-{1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl}-1,3-dihydro-*2H*-benzoimidazol-2-one [*2062-78-4*]

Pimozide contains not less than 98.5% and not more than 101.0% of $C_{28}H_{29}F_2N_3O$.

Description Pimozide occurs as a white to pale yellowish white powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Pimozide in methanol (1 in 25000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Pimozide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 216 - 220ºC.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pimozide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution by using 5 mL of sulfuric acid (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Pimozide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Pimozide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the peak of pimozide from the sample solution is not lager than the peak area of pimozide from the standard solution, and the total area of the peaks other than the peak of pimozide from the sample solution is not larger than 1.5 times of the peak area of pimozide 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 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase A: Dissolve 2.5 g of ammonium acetate and 8.5 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Mobile phase B: Acetonitrile

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 (vol%)	Mobile phase B (vol%)
0 – 10	80 → 70	20 → 30
10 – 15	70	30

Flow rate: 2.0 mL per minute.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 10 mL. Confirm that the peak area of pimozide obtained from 10 μL of this solution is equivalent to 8 to 12% of that of pimozide from the standard solution.

System performance: Dissolve 5 mg of Pimozide and 2 mg of mebendazole in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mebendazole and pimozide 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 pimozide is not more than 2.0%.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105ºC, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 70 mg of dried Pimozide, dissolve in 25 mL of acetic acid for nonaqueous titration, and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

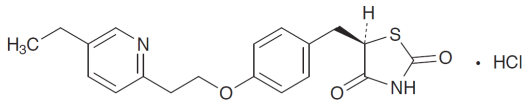
Each mL of 0.02 mol/L perchloric acid VS
= 9.231 mg of $C_{28}H_{29}F_2N_3O$

Containers and storage Containers—Well-closed containers.

Add the following:

Pioglitazone Hydrochloride

ピオグリタゾン塩酸塩



and enantiomer

$C_{19}H_{20}N_2O_3S$·HCl: 392.90
(5*RS*)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}thiazolidine-2,4-dione monohydrochloride [*112529-15-4*]

Pioglitazone Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{19}H_{20}N_2O_3S$·HCl, calculated on the anhydrous basis.

Description Pioglitazone Hydrochloride occurs as white crystals or crystalline powder.

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

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Pioglitazone Hydrochloride in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Pioglitazone Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pioglitazone 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 Pioglitazone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pioglitazone Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Pioglitazone Hydrochloride in 1 mL of nitric acid, and add 4 mL of dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pioglitazone Hydrochloride according to Method 4, and perform the test. After incineration, use 3 mL of hydrobromic acid instead of 3 mL of hydrochloric acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Pioglitazone Hydrochloride in 20 mL of methanol, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 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 exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks, having the relative retention times of about 0.7, about 1.4 and about 3.0 with respect to pioglitazone from the sample solution, is not larger than 2/5 times the peak area of pioglitazone from the standard solution, and the area of each peak other than the peak of pioglitazone and other than those mentioned above is smaller than 1/5 times the peak area of pioglitazone from the standard solution. Furthermore, the total area of the peaks other than the peak of pioglitazone is not larger than the peak area of pioglitazone 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 pioglitazone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pioglitazone obtained from 40 μL of this solution is equivalent to 7 to 13% of that of pioglitazone from the standard solution.

System performance: Dissolve 50 mg of Pioglitazone Hydrochloride in 10 mL of a solution of benzophenone in methanol (1 in 750), and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 40 μL of this solution under the above operating conditions, pioglitazone and benzophenone 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 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 0.2% (0.5 g, coulometric titration). For anolyte solution, use anolyte solution for water determination A.

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Pioglitazone Hydrochloride and Pioglitazone Hydrochloride Reference Standard (separately, determine the water content <*2.48*> in the same manner as Pioglitazone Hydrochloride), add exactly 10 mL of the internal standard solution and methanol to make 100 mL. Pipet 2 mL each of these solutions, add the mobile phase to make 20 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of

pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$·HCl) = $W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of Pioglitazone Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of benzophenone in methanol (1 in 750)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 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 25ºC.

Mobile phase: A mixture of ammonium acetate solution (77 in 10000), acetonitrile and acetic acid (100) (25:25:1)

Flow rate: Adjust the flow rate so that the retention time of pioglitazone 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, pioglitazone 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pipemidic Acid Hydrate

ピペミド酸水和物

Delete the Loss on drying and change the Origin/limits of content and Purity (1) and (2) to read:

Pipemidic Acid Hydrate contains not less than 98.5% and not more than 101.0% of pipemidic acid ($C_{14}H_{17}N_5O_3$: 303.32), calculated on the anhydrous basis.

Description Pipemidic Acid Hydrate 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), and practically insoluble in methanol.

It dissolves in sodium hydroxide TS.

It is gradually colored on exposure to light.

Melting point: about 250ºC (with decomposition).

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute nitric acid, and filter through a glass filter (G3). To 30 mL of the filtrate 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 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <*1.14*>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute hydrochloric acid, and filter through a glass filter (G3). To 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

Add the following next to the Purity:

Water <*2.48*> 14.5 - 16.0% (20 mg, coulometric titration)

Change the Assay to read:

Assay Weigh accurately about 0.35 g of Pipemidic Acid Hydrate, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Add the following:

Pivmecillinam Hydrochloride Tablets

ピブメシリナム塩酸塩錠

Pivmecillinam Hydrochloride Tablets contains not less than 93.0% and not more than 107.0% of the labeled potency of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

Method of preparation Prepare as directed under Tablets, with Pivmecillinam Hydrochloride.

Identification Powder Pivmecillinam Hydrochloride Tablets, dissolve a portion of the powder, equivalent to 35 mg (potency) of Pivmecillinam Hydrochloride according to the labeled amount, in 4 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately dissolve 25 mg of Pivmecillinam Hydrochloride Reference Standard in 2 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and 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 in iodine vapor for 10 minutes: the principal spot obtained from the sample solution has the same R_f value as the spot from the standard solution.

Water *<2.48>* Not more than 3.0% (1g of powdered Pivmecillinam Hydrochloride Tablets, volumetric titration, direct titration).

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Pivmecillinam Hydrochloride Tablets add 40 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 50 mL. Pipet V mL, equivalent to about 10 mg (potency) of Pivmecillinam Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
$= W_S \times (Q_T/Q_S) \times (25/V)$

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

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12500)

Disintegration *<6.09>* Perform the test using the disk: it meets the requirement.

Assay Weigh accurately the mass of not less than 20 Pivmecillinam Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Pivmecillinam Hydrochloride, add 50 mL of the mobile phase, shake vigorously for 10 minutes, and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Pivmecillinam Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Pivmecillinam Hydrochloride.

Amount [mg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
$= W_S \times (Q_T/Q_S) \times 5$

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

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12500)

Containers and storage Containers—Tight containers.

Potato Starch

バレイショデンプン

Change the Identification to read:

Identification (1) Examined under a microscope *<5.01>* using a mixture of water and glycerin (1:1), Potato Starch presents granules, either irregularly shaped, ovoid or pear-shaped, usually 30-100 μm in size but occasionally exceeding 100 μm, or rounded, 10-35 μm in size. There are occasional compound granules having two to four components. The ovoid and pear-shaped granules have an eccentric hilum and the rounded granules acentric or slightly eccentric hilum. All granules show clearly visible concentric striations. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

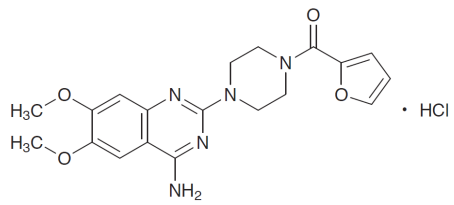
Add the following next to Purity (3):

Purity ◆**(4)** Foreign matter—Under a microscope *<5.01>*, Potato Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.◆

Add the following:

Prazosin Hydrochloride

プラゾシン塩酸塩



$C_{19}H_{21}N_5O_4$·HCl: 419.86
1-(4-Amino-6,7-dimethoxy-quinazolin-2-yl)-4-(2-furoyl)piperazine monohydrochloride [*19237-84-4*]

Prazosin Hydrochloride, when dried, contains not less than 97.0% and not more than 103.0% of $C_{19}H_{21}N_5O_4$·HCl.

Description Prazosin Hydrochloride occurs as a white crystalline powder.

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

It gradually turns pale yellowish white on exposure to light.

Melting point: about 270ºC (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Prazosin Hydrochloride in 0.01 mol/L hydrochloric acid-methanol TS (1 in 200000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Prazosin 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 Prazosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Prazosin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Prazosin Hydrochloride add 5 mL of water and 1 mL of ammonia TS, shake, allow to stand for 5 minutes, and filter. Render the filtrate acid with acetic acid (100): the solution responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Prazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Prazosin Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prazosin from the sample solution is not larger than 2 times the peak area of prazosin from the standard solution, and the total area of the peaks other than the peak of prazosin from the sample solution is not larger than 5 times the peak area of prazosin from the standard solution.

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 25ºC.

Mobile phase: Dissolve 3.484 g of sodium 1-pentane sulfonate and 18 mL of tetramethylammonium hydroxide in 900 mL of water, adjust the pH to 5.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 1000 mL of methanol

Flow rate: Adjust the flow rate so that the retention time of prazosin is about 9 minutes.

Time span of measurement: About 6 times as long as the retention time of prazosin.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of prazosin obtained from 20 μL of this solution is equivalent to 35 to 65% of that of prazosin from 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 factor of the peak of prazosin are not less than 4000 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 prazosin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 25 mg each of Prazosin Hydrochloride and Prazosin Hydrochloride Reference Standard, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 3 mL each of these solutions, and add a mixture of methanol and water (7:3) to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, A_T and A_S, of prazosin in each solution.

Amount (mg) of prazosin hydrochloride ($C_{19}H_{21}N_5O_4 \cdot HCl$) $= W_S \times (A_T/A_S)$

W_S: Amount (mg) of Prazosin 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 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25ºC.

Mobile phase: A mixture of methanol, water, acetic acid (100) and diethylamine (3500: 1500: 50:1)

Flow rate: Adjust the flow rate so that the retention time of prazosin 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 and the symmetry factor of the peak of prazosin are not less than 5000 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 prazosin

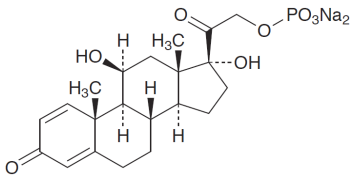
is not more than 1.0%.

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Add the following:

Prednisolone Sodium Phosphate

プレドニゾロンリン酸エステルナトリウム



$C_{21}H_{27}Na_2O_8P$: 484.39
Disodium 11*β*,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-phosphate [*125-02-0*]

Prednisolone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of $C_{21}H_{27}Na_2O_8P$, calculated on the anhydrous basis.

Description Prednisolone Sodium Phosphate occurs as a white to pale yellow powder.

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

It is hygroscopic.

Identification (1) Moisten 1.0 g of Prednisolone Sodium Phosphate with a small amount of sulfuric acid, and gradually heat to incinerate. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests <*1.09*> for phosphate.

(2) Dissolve 2 mg of Prednisolone Sodium Phosphate in 2 mL of sulfuric acid, and allow to stand for 2 minutes: a deep red color, without fluorescence, develops.

(3) Determine the absorption spectrum of a solution of Prednisolone Sodium Phosphate (1 in 50000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Prednisolone Sodium Phosphate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) The solution obtained in (1) responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +96 - +103° (1 g, calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Prednisolone Sodium Phosphate in 100 mL of water: the pH of the solution is between 7.5 and 9.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Prednisolone Sodium Phosphate in 10 mL of water: the solution is clear and not more colored than the following control solution

Control solution: To a mixture of 3.0 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 3.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 2.4 mL of Copper (II) Sulfate Colorimetric Stock Solution add diluted hydrochloric acid (1 in 40) to make 10 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 0.5 g of Prednisolone Sodium Phosphate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.25 g of Prednisolone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution and Phosphoric Acid Standard Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphtol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20±1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of each solution from the sample solution and standard solution at 740 nm: the content of free phosphoric acid is not more than 1.0%

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = (1/W) \times (A_T/A_S) \times 257.8$$

W: Amount (mg) of Prednisolone Sodium Phosphate, calculated on the anhydrous basis.

(4) Related substances—Dissolve 10 mg of Prednisolone Sodium Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of prednisolone phosphate from the sample solution is not larger than 1.5 times the peak area of prednisolone phosphate from the standard solution, and the total area of the peaks other than the peak of prednisolone phosphate from the sample solution is not larger than 2.5 times the peak area of prednisolone phosphate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 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 (3 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid. To 1000 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of prednisolone phosphate is about 7 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of prednisolone phosphate obtained from 20 μL of this solution is equivalent to 7 to 13% of that of prednisolone phosphate from 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 factor of the peak of prednisolone phosphate are not less than 3000 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 prednisolone phosphate is not more than 2.0%.

(5) Residual solvent—Being specified separately.

Water <*2.48*> Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Prednisolone Sodium Phosphate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add 1 mL of alkaline phosphatase TS, and allow to stand for 2 hours with occasional shaking. To this solution add exactly 20 mL of 1-octanol, and shake vigorously. Centrifuge this solution, pipet 10 mL of the 1-octanol layer, add 1-octanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Reference Standard, previously dried at 105ºC for 3 hours, and dissolve in 1-octanol to make exactly 100 mL. Pipet 6 mL of this solution, add a solution prepared by adding 1 mL of alkaline phosphatase TS to 2 mL water and being allowed to stand for 2 hours with occasional gentle shaking, add exactly 14 mL of 1-octanol, and shake vigorously. Proceed in the same manner as the sample solution to make the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 1-octanol as the blank, and determine the absorbances, A_T and A_S, at 245 nm.

Amount (mg) of prednisolone sodium phosphate ($C_{21}H_{27}Na_2O_8P$)
$= W_S \times (A_T/A_S) \times 3 \times 1.3439$

W_S: Amount (mg) of Prednisolone Reference Standard

Containers and storage Containers—Tight containers.

Probenecid Tablets

プロベネシド錠

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Probenecid Tablets add 30 mL of water and 2 mL of 1 mol/L hydrochloric acid TS, treat with ultrasonic waves with occasional shaking to disintegrate the tablet completely, and add ethanol (99.5) to make exactly 100 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, and add ethanol (99.5) to make exactly V mL so that each mL contains about 15 μg of probenecid ($C_{13}H_{19}NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of Probenecid Reference Standard, previously dried at 105ºC for 4 hours, dissolve in 15 mL of water, 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 3 mL of this solution, and add 1 mL of 1 mol/L hydrochloric acid TS and ethanol (99.5) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared by adding ethanol (99.5) to 1 mL of 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, as the blank, and determine the absorbances, A_T and A_S, at 248 nm.

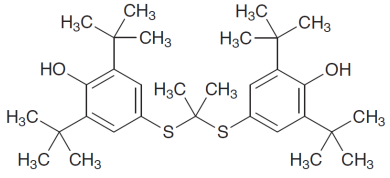
Amount (mg) of probenecid ($C_{13}H_{19}NO_4S$)
$= W_S \times (A_T/A_S) \times (V/25)$

W_S: Amount (mg) of Probenecid Reference Standard

Add the following:

Probucol

プロブコール



$C_{31}H_{48}O_2S_2$: 516.84
4,4′-[Propan-2,2-diylbis(sulfandiyl)]bis[2,6-bis(1,1-dimethylethyl)phenol] [*23288-49-5*]

Probucol, when dried, contains not less than 98.5% and not more than 101.0% of $C_{31}H_{48}O_2S_2$.

Description Probucol occurs as a white crystalline powder.

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

It gradually turns light yellow on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Probucol in methanol (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probucol 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 Probucol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Probucol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 125 - 128ºC.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Probucol 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—Conduct this procedure using light-resistant vessels. Dissolve 0.40 g of Probucol in 5 mL of ethanol (99.5), add the mobile phase to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 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 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak having the relative retention time of about 0.9 with respect to probucol from the sample solution is not larger than the peak area of probucol from the standard solution; the area of peak having the relative retention time of about 1.9 with respect to probucol from the sample solution is no larger than 25 times the peak area of probucol from the standard solution; and the area of each peak other than the peak of probucol and other than the peaks mentioned above is not larger than 5 times the peak area of probucol from the standard solution. Furthermore, the total area of the peaks other than probucol from the sample solution is not larger than 50 times the peak area of probucol from the standard solution. For this calculation, use the areas of the peaks, having the relative retention times of about 0.9 and about 1.9 with respect to probucol, after multiplying by their relative response factors, 1.2 and 1.4, 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 3 times as long as the retention time of probucol, beginning after the solvent peak, excluding the peak having the relative retention time of about 0.5 with respect to probucol.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of probucol obtained from 5 μL of this solution is equivalent to 14 to 26% of that of probucol from the standard solution.

System performance: To 1 mL of the sample solution add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of a solution of phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) in the mobile phase (1 in 1000), 5 mL of ethanol (99.5), and the mobile phase to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) and probucol 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of probucol is not more than 5%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 80ºC, 1 hour).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg each of Probucol and Probucol Reference Standard, previously dried, dissolve each in 5 mL of tetrahydrofuran, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of probucol to that of the internal standard.

$$\text{Amount (mg) of probucol } (C_{31}H_{48}O_2S_2) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Probucol Reference Standard

Internal standard solution—Dissolve 0.2 g of phthalic acid bis(cis-3,3,5-trimethylcyclohexyl) in 1 mL of tetrahydrofuran, and add the mobile phase to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 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 40ºC.

Mobile phase: A mixture of acetonitrile and water (93:7)

Flow rate: Adjust the flow rate so that the retention time of probucol is about 13 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 solution and probucol 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 ratio of the peak area of probucol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Procainamide Hydrochloride

プロカインアミド塩酸塩

Change the Origin/limits of contents, Description, Identification, Purity and Loss on drying to read:

Procainamide Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of $C_{13}H_{21}N_3O$·HCl.

Description Procainamide Hydrochloride occurs as a white to light yellow crystalline powder.

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

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Procainamide Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Procainamide 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 <*1.11*>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Procainamide Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase 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 standard solution as directed under Liquid Chromatography <*2.01*> 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 procainamide from the sample solution is not larger than the peak area of procainamide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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 40ºC.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and methanol (9:1)

Flow rate: Adjust the flow rate so that the retention time of procainamide is about 9 minutes.

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

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of procainamide obtained from 10 μL of this solution is equivalent to 40 to 60% of that of procainamide from 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 factor of the peak of procainamide are not less than 10000 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 procainamide is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.3% (2 g, 105ºC, 4 hours).

Procainamide Hydrochloride Injection

プロカインアミド塩酸塩注射液

Change the Origin/limits of content and Identification to read:

Procainamide Hydrochloride 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 procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl: 271.79).

Identification **(1)** To a volume of Procainamide Hydrochloride Injection, equivalent to 10 mg of Procainamide Hydrochloride according to the labeled amount, add 1 mL of dilute hydrochloric acid and water to make 5 mL: the solution responds to the Qualitative Tests <*1.09*> (1) for primary aromatic amines.

(2) To a volume of Procainamide Hydrochloride Injection, equivalent to 0.1 g of Procainamide Hydrochloride according to the labeled amount, add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. Determine the absorption

spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 281 nm.

(3) Procainamide Hydrochloride Injection responds to the Qualitative Tests <*1.09*> (2) for chloride.

Add the following next to the Identification:

Bacterial endotoxins <*4.01*> Less than 0.30 EU/mg.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Procainamide Hydrochloride Tablets

プロカインアミド塩酸塩錠

Change the Origin/limits of content and Identification to read:

Procainamide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl: 271.79).

Identification To a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride according to the labeled amount, add 30 mL of water, shake well, filter, and use the filtrate as the sample solution. To 0.2 mL of the sample solution add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

Add the following next to the Identification:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Procainamide Hydrochloride Tablets add $3V/5$ mL of 0.02 mol/L phosphate buffer solution, pH 3.0, treat with ultrasonic waves to disintegrate the tablet completely, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly V mL so that each mL contains about 2.5 mg of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl), and shake for 5 minutes. Centrifuge this solution, pipet 1 mL of the supernatant liquid, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make 250 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl)
$= W_S \times (A_T/A_S) \times (V/20)$

W_S: Amount (mg) of procainamide hydrochloride for assay

Change the Dissolution and Assay to read:

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Procainamide Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Procainamide Hydrochloride Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 7 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105ºC for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 278 nm.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/2)$

W_S: Amount (mg) of procainamide hydrochloride for assay
C: Labeled amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl) in 1 tablet

Assay To 10 Procainamide Hydrochloride Tablets add 300 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, and treat with ultrasonic waves to disintegrate the tablets completely. To this solution add 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 500 mL, and stir for 5 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, and add 0.02 mol/L phosphate buffer solution, pH 3.0, to make V' mL so that each mL contains about 10 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of procainamide hydrochloride for assay, previously dried at 105ºC for 4 hours, dissolve in 0.02 mol/L phosphate buffer solution, pH 3.0, to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L phosphate buffer solution, pH 3.0, to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, A_T and A_S, of procainamide in each solution.

Amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$·HCl)

$= W_S \times (A_T/A_S) \times (V'/V) \times (1/10)$

W_S: Amount (mg) of procainamide hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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 40ºC.

Mobile phase: A mixture of 0.02 mol/L phosphate buffer solution, pH 3.0, and methanol (9:1)

Flow rate: Adjust the flow rate so that the retention time of procainamide 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, the number of theoretical plates and the symmetry factor of the peak of procainamide are not less than 10000 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 procainamide is not more than 1.0%.

Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Add the following next to the Identification:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Prochlorperazine Maleate Tablets add $3V/5$ mL of a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves until the tablet is disintegrated, and shake vigorously for 10 minutes. Add exactly $V/20$ mL of the internal standard solution, and a mixture of dilute phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make V mL so that each mL contains about 80 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$), and use this solution as the sample solution. Centrifuge this solution, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$)
$= W_S \times (Q_T/Q_S) \times (V/250)$

W_S: Amount (mg) of Prochlorperazine Maleate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000).

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Prochlorperazine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Prochlorperazine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 9 μg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of prochlorperazine maleate for assay, previously dried at 105ºC for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using the dissolution medium as the blank, and determine the absorbances, A_T and A_S, at 255 nm.

Dissolution rate (%) with respect to the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$

W_S: Amount (mg) of Prochlorperazine Maleate Reference Standard

C: Labeled amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$) in 1 tablet

Change the Assay to read:

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Prochlorperazine Maleate Tablets, and powder in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 8 mg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), and shake vigorously for 10 minutes. Add exactly 5 mL of the internal standard solution, and add a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Prochlorperazine Maleate Reference Standard, previously dried at 105ºC for 3 hours, and dissolve in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of prochlorperazine to that of the internal standard.

Amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$)
$= W_S \times (Q_T/Q_S) \times (2/5)$

W_S: Amount (mg) of Prochlorperazine Maleate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 1000)

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 25ºC.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (11:9)

Flow rate: Adjust the flow rate so that the retention time of prochlorperazine 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, prochlorperazine 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 ratio of the peak area of prochlorperazine to that of the internal standard is not more than 1.0%.

Progesterone

プロゲステロン

Change the Description, Identification, Optical rotation, Melting point, Purity and Assay to read:

Description Progesterone occurs as white crystals or a white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Progesterone in ethanol (99.5) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Progesterone 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 Progesterone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Progesterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone Reference Standard in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +184 - +194º (after drying, 0.2 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <*2.60*> 128 - 133ºC or 120 - 122ºC.

Purity Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this 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 Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) 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 obtained from the sample solution is not more intense than the spot from the standard solution.

Assay Weigh accurately about 10 mg each of Progesterone and Progesterone Reference Standard, previously dried, and dissolve each in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (99.5) to make exactly 50 mL, and use these solution as the sample solution and standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of progesterone ($C_{21}H_{30}O_2$) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of Progesterone Reference Standard

Progesterone Injection

プロゲステロン注射液

Change the Origin/limits of content and Identification to read:

Progesterone Injection is an oily solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

Identification To 1 mL of Progesterone Injection add 1 mL of diluted ethanol (9 in 10), shake well, take the ethanol layer, shake well with 1 mL of petroleum benzin, and use the ethanol layer as the sample solution. Separately, dissolve about 5 mg of Progesterone Reference Standard in 1 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and diethylamine (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat the plate at 105ºC for 10 minutes: the principal spot obtained from the sam-

ple solution has the same R_f value as the spot from the standard solution.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Direct method: it meets the requirement.

Change the Assay to read:

Assay Measure the specific gravity of Progesterone Injection. Weigh accurately the mass of Progesterone Injection, equivalent to about 1 mL, mix with 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly *V* mL so that each mL contains about 0.5 mg of progesterone ($C_{21}H_{30}O_2$). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Progesterone Reference Standard, previously dried in vacuum for 4 hours using phosphorus (V) oxide as the desiccant, dissolve in 2 mL of tetrahydrofuran, and add ethanol (99.5) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of progesterone to that of the internal standard.

$$\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) = W_S \times (Q_T/Q_S) \times (V/20)$$

W_S: Amount (mg) of Progesterone Reference Standard

Internal standard solution—A solution of testosterone propionate in ethanol (99.5) (1 in 4000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 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 35ºC.

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

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

System suitability—

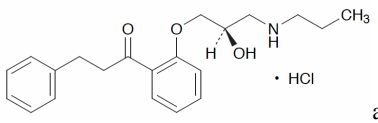
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, progesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of progesterone to that of the internal standard is not more than 1.0%.

Add the following:

Propafenone Hydrochloride

プロパフェノン塩酸塩



and enantiomer

$C_{21}H_{27}NO_3$·HCl: 377.90

1-{2-[(2*RS*)-2-Hydroxy-3-(propylamino)propyloxy]phenyl}-3-phenylpropan-1-one monohydrochloride [*34183-22-7*]

Propafenone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{21}H_{27}NO_3$·HCl.

Description Propafenone Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

A solution of Propafenone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification **(1)** Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 3 mL of this solution add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Propafenone Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Propafenone Hydrochloride in 20 mL of water by warming. After cooling, to 10 mL of this solution add 1 mL of dilute nitric acid, and filter to separate formed precipitate : the filtrate responds to the Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*> 172 - 175ºC.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Propafenone 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 0.10 g of Propafenone Hydrochloride in 20 mL of the mobile phase in the operating conditions 1, and use this solution as the sample solution. Pipet 2 mL of this solution, and add the mobile phase in the operating conditions 1 to make exactly 50 mL. Pipet 2.5 mL of this solu-

tion, add 2.5 mL of a solution of diphenyl phthalate in methanol (1 in 2000), add the mobile phase in the operating conditions 1 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions 1 and 2. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of propafenone from the sample solution is not larger than the peak area of propafenone from the standard solution.

Operating conditions 1—

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 40ºC.

Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 39 minutes.

Time span of measurement: Beginning after the solvent peak to the retention time of diphenyl phthalate.

System suitability 1—

System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 1, propafenone and isopropyl benzoate 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 1, the relative standard deviation of the peak area of propafenone is not more than 2.0%.

Operating conditions 2—

Detector, column and column temperature: Proceed as directed in the operation conditions 1.

Mobile phase: Dissolve 7.33 g of sodium 1-decanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 700 mL of the filtrate add 700 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of diphenyl phthalate is about 11 minutes.

Time span of measurement: About 2.5 times as long as the retention time of diphenyl phthalate.

System suitability 2—

System performance: Dissolve 12 mg of Propafenone Hydrochloride and 50 mg of isopropyl benzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions 2, propafenone and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 21.

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 propafenone is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g,105ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propafenone Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS = 18.90 mg of $C_{21}H_{27}NO_3$·HCl.

Containers and storage Containers—Well-closed containers.

Add the following:

Propafenone Hydrochloride Tablets

プロパフェノン塩酸塩錠

Propafenone Hydrochloride Tablets contain not less than 96.0% and not more than 104.0% of the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl: 377.90).

Method of preparation Prepare as directed under Tablets, with Propafenone Hydrochloride.

Identification To a quantity of Propafenone Hydrochloride Tablets, equivalent to 0.3 g of Propafenone Hydrochloride according to the labeled amount, add 60 mL of water, and disintegrate by warming. After cooling, centrifuge, and to 3 mL of the supernatant liquid add water to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 247 nm and 251 nm, and between 302 nm and 306 nm. Separately, determine the both maximal absorbances, A_1 and A_2, of the solution,: the ratio of A_1/A_2 is between 2.30 and 2.55.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propafenone Hydrochloride Tablets add 30 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 6 mg of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl), add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl)

$= W_S \times (Q_T/Q_S) \times (10/V)$

W_S: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200)

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Propafenone Hydrochloride is not less than 75%.

Start the test with 1 tablet of Propafenone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 67 μg of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 13 mg of propafenone hydrochloride for assay, previously dried at 105ºC for 2 hours, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Dissolution rate (%) with respect to the labeled amount of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 450$

W_S: Amount (mg) of propafenone hydrochloride for assay
C: Labeled amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl) in 1 tablet

Assay To a quantity of Propafenone Hydrochloride Tablets, equivalent to 1.5 g of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl), add 70 mL of a mixture of water and acetonitrile (1:1), shake well to disintegrate, shake well for another 5 minutes, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and centrifuge. Pipet 4 mL of the supernatant liquid, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of propafenone hydrochloride for assay, previously dried at 105ºC for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of propafenone to that of the internal standard.

Amount (mg) of propafenone hydrochloride ($C_{21}H_{27}NO_3$·HCl)
$= W_S \times (Q_T/Q_S) \times 50$

W_S: Amount (mg) of propafenone hydrochloride for assay

Internal standard solution—A solution of isopropyl benzoate in methanol (1 in 200)

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 40ºC.

Mobile phase: Dissolve 4.6 g of sodium 1-nonanesulfonate and 2.3 g of phosphoric acid in water to make 1000 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 900 mL of the filtrate add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of propafenone 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, propafenone 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 ratio of the peak area of propafenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Propylthiouracil Tablets

プロピルチオウラシル錠

Add the following next to Identification:

Uniformity of dosage units *<6.02>* Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propylthiouracil Tablets add $3V/4$ mL of 2nd fluid for dissolution test, treat with ultrasonic waves until the tablet is disintegrated, and add 2nd fluid for dissolution test to make exactly V mL so that each mL contains about 0.25 mg of propylthiouracil ($C_7H_{10}N_2OS$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$= W_S \times (A_T/A_S) \times (V/200)$

W_S: Amount (mg) of propylthiouracil for assay

Change the Dissolution and Assay to read:

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Propylthiouracil Tablets is not less than 80%.

Start the test with 1 tablet of Propylthiouracil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the dissolution medium to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh about 50 mg of propylthiouracil for assay, previously dried at 105ºC for 2 hours, and dissolve in the dissolution medium to make exactly 1000 mL. Pipet 5 mL of this solution, add the dissolution medium to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$)
$= W_S \times (A_T/A_S) \times (1/C) \times 90$

W_S: Amount (mg) of propylthiouracil for assay
C: Labeled amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Propylthiouracil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of propylthiouracil ($C_7H_{10}N_2OS$), add 150 mL of 2nd fluid for dissolution test, disperse finely the particles with the aid of ultrasonic waves, and add 2nd fluid for dissolution test to make exactly 200 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propylthiouracil for assay, previously dried at 105ºC for 2 hours, and dissolve in 2nd fluid for dissolution test to make exactly 200 mL. Pipet 2 mL of this solution, add 2nd fluid for dissolution test to make 100 mL, and use this solution as the standard solution. Determine the absorbance at 274 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of propylthiouracil for assay

Protamine Sulfate Injection

プロタミン硫酸塩注射液

Delete the Purity and Heparin-binding capacity, and change the Origin/limits of content to read:

Protamine Sulfate Injection is an aqueous solution for injection.

It contains not less than 92.0% and not more than 108.0% of the labeled amount of Protamine Sulfate. It binds with not less than 100 Units of heparin per mg of the labeled amount.

Identification (1) Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

Add the following next to the pH:

Bacterial endotoxins <*4.01*> Less than 6.0 EU/mg.

Add the following next to the Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Protein—Pipet a volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate, transfer to a Kjeldahl flask, evaporate on a water bath to dryness with the aid of a current of air, determine the nitrogen as directed under Nitrogen Determination <*1.08*>, and calculate the amount of protein by converting 0.24 mg of nitrogen (N: 14.01) to 1 mg of protein.

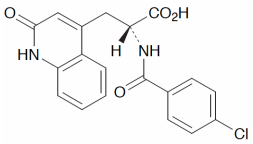
(2) Heparin-binding activity—Proceed the test as directed in the Heparin-binding capacity under Protamine Sulfate, changing the sample solution (a) as below, and determine the amount of heparin bound to 1 mg of protein by dividing by the amount of protein.

(i) Sample solution (a)—Pipet a volume of Protamine Sulfate Injection, equivalent to 15.0 mg of Protamine Sulfate according to the labeled amount, and add water to make exactly 100 mL. Repeat this procedure two more times, and designate the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

Add the following:

Rebamipide

レバミピド



and enantiomer

$C_{19}H_{15}ClN_2O_4$: 370.79

(2*RS*)-2-(4-Chlorobenzoylamino)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid [*90098-04-7*]

Rebamipide, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{15}ClN_2O_4$.

Description Rebamipide occurs as a white crystalline powder, and has a bitter taste.

It is soluble in *N*,*N*-dimethylformamide, very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Rebamipide in *N*,*N*-dimethylformamide (1 in 20) shows no optical rotation.

Melting point: about 291ºC (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Rebamipide in methanol (7 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Rebamipide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Rebamipide as directed under Flame Coloration Test <*1.04*>(2): a green color appears.

Purity **(1)** Chloride <*1.03*>—Dissolve 0.5 g of Rebamipide in 40 mL of *N*,*N*,-dimethylformamide, 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 0.40 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of *N*,*N*,-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Rebamipide 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) Rebamipide *m*-chloro isomer Dissolve 40 mg of Rebamipide in a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of this solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak of rebamipide *m*-chloro isomer, having the relative retention time of about 0.95 with respect to rebamipide, from the sample solution, is not larger than 3/8 times the area of the peak of rebamipide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 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 25ºC.

Mobile phase: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 minutes.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make exactly 25 mL. Confirm that the peak area of rebamipide obtained from 10 μL of this solution is equivalent to 15 to 25% of that of rebamipide from 10 μL of the standard solution.

System performance: To 1 mL of the sample solution add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0 and methanol (7:7:6) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rebamipide are not less than 11000 and not more than 1.2, 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 rebamipide is not more than 2.0%.

(4) Related substances— Perform the test with exactly 10 μL each of the sample solution and standard solution obtained in (3) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks of rebamipide *o*-chloro isomer and debenzoylated isomer, having the relative retention times of about 0.5 and about 0.7, respectively, with respect to rebamipide from the sample solution, is not larger than 3/8 times the peak area of rebamipide from the standard solution, the area of each peak other than the peak mentioned above is not larger than 1/4 times the peak area of rebamipide from the standard solution, and the total area of the peaks other than rebamipide from the sample solution is not larger than the peak area of rebamipide from the standard solution. For the calculation, use the peak area of rebamipide

o-chloro isomer, after multiplying by the response factor, 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength : 232 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 40ºC.

Mobile phase: Dissolve 2.44 g of sodium 1-decanesulfonate in 1000 mL of water and to this solution add 1000 mL of methanol and 10 mL of phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 12 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make exactly 50 mL. Confirm that the peak area of rebamipide obtained from 10 μL of this solution is equivalent to 7 to 13% of that of rebamipide from 10 μL of the standard solution.

System performance: Dissolve 20 mg of 4-chlorobenzoate in methanol to make 50 mL. To 5 mL each of this solution and the sample solution add a mixture of water, 0.05 mol/L phosphate buffer solution, pH 6.0, and methanol (7:7:6) to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, rebamipide and 4-chlorobenzoate 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 rebamipide is not more than 2.0%.relative response

(5) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 3.0% (1 g, 105ºC, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Rebamipide, previously dried, dissolve in 60 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide VS until the color of the solution changes from pale yellow to colorless(indicator: 2 drops of phenol red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide VS
= 37.08 mg of $C_{19}H_{15}ClN_2O_4$

Containers and storage Containers—Well-closed containers. Storage—Light-resistant.

Add the following:

Rebamipide Tablets

レバミピド錠

Rebamipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of rebamipide ($C_{19}H_{15}ClN_2O_4$: 370,79).

Method of preparation Prepare as directed under Tablets, with Rebamipide.

Identification To a quantity of powdered Rebamipide Tablets, equivalent to 30 mg of Rebamipide according to the labeled amount, add 5 mL of a mixture of methanol and ammonia solution (28) (9:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 30 mg of rebamipide for assay in 5 mL of a mixture of methanol and ammonia solution (28) (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and formic acid (75:25:2) 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.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Rebamipide Tablets add 10 mL of water, shake well for 10 minutes, add exactly 10 mL of the internal standard solution, add 10 mL of *N*,*N*-dimethylformamide, shake well for 5 minutes, and add *N*,*N*-dimethylformamide to make 50 mL. Centrifuge this solution, pipet *V* mL of the supernatant liquid, equivalent to 3 mg of rebamipide ($C_{19}H_{15}ClN_2O_4$), and add 20 mL of *N*,*N*-dimethylformamide and water to make 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105ºC for 2 hours, dissolve in *N*,*N*-dimethylformamide, and add exactly 10 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 50 mL. Pipet 1.5 mL of this solution, add 20 mL of *N*,*N*-dimethylformamide, add water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= W_S \times (Q_T/Q_S) \times (3/2V)$

W_S: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in *N*,*N*-dimethylformamide (1 in 150)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using 900 mL of diluted disodium hydrogen phosphate-citric acid buffer solution (1 in 4), pH 6.0, as the dissolution medium, the dissolution rate in 60 minutes of Rebamipide Tablets is not less than 75%.

Start the test with 1 tablet of Rebamipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 22 μg of rebamipide ($C_{19}H_{15}ClN_2O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of rebamipide for assay, previously dried at 105ºC for 2 hours, and dissolve in *N*,*N*-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the blank, and determine the absorbances, A_T and A_S, at 326 nm.

Dissolution rate (%) with respect to the labeled amount of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of rebamipide for assay
C: Labeled amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$) in 1 tablet

Assay To 10 Rebamipide Tablets add exactly V/5 mL of the internal standard solution and 50 mL of *N*,*N*-dimethylformamide, and disintegrate the tablets with the aid of ultrasonic waves. Shake this solution for 5 minutes, add *N*,*N*-dimethylformamide to make V mL so that each mL contains about 10 mg of rebamipide ($C_{19}H_{15}ClN_2O_4$). Centrifuge this solution, and to 5 mL of the supernatant liquid add *N*,*N*-dimethylformamide to make 50 mL. To 2 mL of this solution add 20 mL of *N*,*N*-dimethylformamide and water to make 50 mL. Filter, if necessary, through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of rebamipide for assay, previously dried at 105ºC for 2 hours, dissolve in *N*,*N*-dimethylformamide, and add exactly 2 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 100 mL. To 2 mL of this solution, add 20 mL of *N*,*N*-dimethylformamide and 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of rebamipide to that of the internal standard.

Amount (mg) of rebamipide ($C_{19}H_{15}ClN_2O_4$)
$= W_S \times (Q_T/Q_S) \times (V/100)$

W_S: Amount (mg) of rebamipide for assay

Internal standard solution—A solution of acetanilide in *N*,*N*-dimethylformamide (1 in 20)

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 25ºC.

Mobile phase: To 300 mL of phosphate buffer solution, pH 6.2, add 750 mL of water. To 830 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rebamipide is about 20 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 rebamipide 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 ratio of the peak area of rebamipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Rice Starch

コメデンプン

Delete the Description and Total ash, and change the Definition to read:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Rice starch consists of the starch granules obtained from the caryopsis of *Oryza sativa* Linné (*Gramineae*).

Add the following next to Definition:

◆**Description** Rice Starch occurs as a white mass or powder. It is practically insoluble in water and in ethanol (99.5).◆

Change the Identification to read:

Identification **(1)** Examined under a microscope <*5.01*> using a mixture of water and glycerin (1:1), Rice Starch presents polyhedral, simple grains 1-10 μm, mostly 4-6 μm, in size. These simple grains often gather in ellipsoidal, compound grains 50-100 μm in diameter. The granules have a poorly visible central hilum and there are no concentric striations. Between orthogonally orientated polarizing plates or prisms, the starch granules show a distinct black cross intersecting at the hilum.

(2) To 1 g of Rice Starch add 50 mL of water, boil for 1 minute, and allow to cool: a thin, cloudy mucilage is formed.

(3) To 1 mL of the mucilage obtained in (2) add 0.05 mL of

diluted iodine TS (1 in 10): an orange-red to dark-blue color is produced which disappears on heating.

Add the following next to the Identification:

pH *<2.54>* To 5.0 g of Rice Starch add 25 mL of freshly boiled and cooled water, and mix gently for 1 minute to achieve suspension. Allow to stand for 15 minutes: the pH of the solution is 5.0 to 8.0.

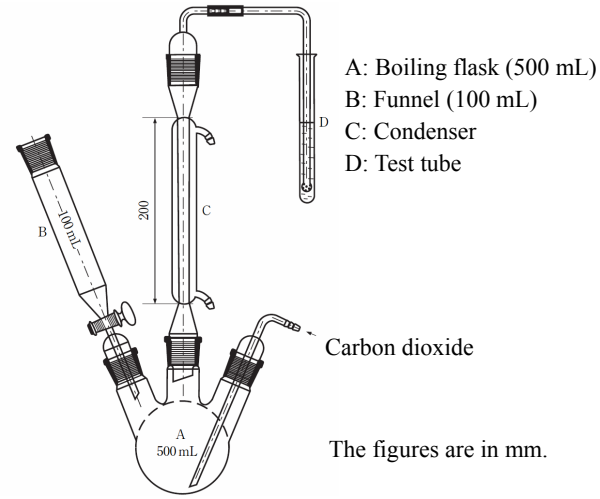
Change the Purity and Loss on drying to read:

Purity **(1)** Iron—To 1.5 g of Rice Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use this solution as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Add ammonia solution (28) to these solutions until the color of a litmus paper to change from red to blue, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Rice Starch add 50 mL of water, shake for 5 minutes, and centrifuge. To 30 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes in the dark. Add 1 mL of starch TS, and titrate *<2.50>* with 0.002 mol/L sodium thiosulfate VS until the starch-iodine color disappears. Perform a blank determination in the same manner, and make any necessary correction. Not more than 1.4 mL of 0.002 mol/L sodium thiosulfate VS is required (not more than 20 ppm, calculated as H_2O_2).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Rice Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat on a water-bath for 15 minutes and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate *<2.50>* with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = (V/W) \times 1000 \times 3.203$$

W: Amount (g) of the sample
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

◆**(4)** Foreign matter—Under a microscope *<5.01>*, Rice Starch does not contain starch granules of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.◆

Loss on drying *<2.41>* Not more than 15.0% (1 g, 130ºC, 90 minutes).

Add the following next to the Loss on drying:

Residue on ignition *<2.44>* Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers.◆

Rokitamycin Tablets

ロキタマイシン錠

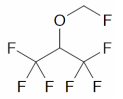
Add the following next to Containers and storage:

Expiration date: 24 months after preparation.

Add the following:

Sevoflurane

セボフルラン



$C_4H_3F_7O$:200.05
1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane
[*28523-86-6*]

Sevoflurane contains not less than 99.0% and not more than 101.0% of $C_4H_3F_7O$, calculated on the anhydrous basis.

Description Sevoflurane is a clear, colorless, and mobile liquid.

It is very slightly soluble in water.
It is miscible with ethanol (99.5).
It is volatile and inflammable.
Refractive index n_D^{20} : 1.2745 – 1.2760
Boiling point: about 58.6°C

Identification Transfer about 1 μL of Sevoflurane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Sevoflurane Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <*2.56*> d_{20}^{20} : 1.510 - 1.530

Purity **(1)** Acidity or alkalinity— To 50 mL of Sevoflurane with 50 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer and use this solution as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Soluble fluoride—To 6 g of Sevoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of diluted 0.01 mol/L sodium hydroxide solution (1 in 20) layer into a Nessler tube. Add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, transfer 0.2 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) into a Nessler tube, and add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH4.3 and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 1 ppm).

Fluorine standard solution: Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(3) Related substances—Perform the test with 2 μL of Sevoflurane as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine each peak area by the automatic integration method and calculate the amount of them by the area percentage method: the amount of the peak of hexafluoroisopropyl methyl ether, having the relative retention time of about 0.84 with respect to sevoflurane, is not more than 0.005%, the amount of each peak other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.0025%, and the total amount of the peaks other than the peaks of sevoflurane and hexafluoroisopropyl methyl ether is not more than 0.005%.

Operating conditions—

Detector, column, injection port temperature, detector temperature, carrier gas and split ratio: Proceed as directed in the operating conditions in the Assay.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 10 minutes, raise at a rate of 10°C per minute to 200°C, and maintain at a constant temperature of about 200ºC.

Flow rate: Adjust the flow rate so that the retention time of sevoflurane is about 7 minutes.

Time span of measurement: About 6 times as long as the retention time of sevoflurane.

System suitability—

Test for required detectability: To 20 μL of Sevoflurane add *o*-xylene to make 20 mL. To 1 mL of this solution add *o*-xylene to make 20 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test and add *o*-xylene to make exactly 10 mL. Confirm that the peak area of sevoflurane obtained from 2 μL of this solution is equivalent to 7 to 13% of the peak area of sevoflurane from the solution for system suitability test.

System performance: When the procedure is run with 2 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of sevoflurane are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 2 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of Sevoflurane is not more than 5.0%.

(4) Residual solvent—Being specified separately.

(5) Residue on evaporation—Evaporate 10 mL of Sevoflurane, exactly measured, on a water bath to dryness, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.

Water <*2.48*> 0.04 - 0.2 w/v% (5 mL, volumetric titration, direct titration).

Assay Pipet 5 mL each of Sevoflurane and Sevoflurane Reference Standard (separately determine the water content <*2.48*> using the same manner as Sevoflurane), to each add exactly 5 mL of dimethoxymethane as an internal standard, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sevoflurane to that of the internal standard.

$$\text{Amount (mg) of sevoflurane } (C_4H_3F_7O) = V_S \times (Q_T / Q_S) \times 1000 \times 1.521$$

V_S: Amount (mL) of Sevoflurane Reference Standard, calculated on the anhydrous basis.

1.521: Specific gravity of Sevoflurane (d_{20}^{20})

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with cyanopropyl methylphenyl silicone for gas chromatography in 1.8 μm thickness.

Column temperature: 40°C

Injection port temperature: A constant temperature of about 200°C.

Detector temperature: A constant temperature of about 225°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of Sevoflurane is about 3 minutes.

Split ratio: 1:20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, sevoflurane and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

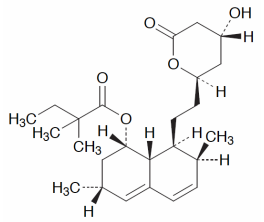
System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sevoflurane to that of the internal standard is not more than 1.0 %.

Containers and storage Containers—Tight containers.

Add the following:

Simvastatin

シンバスタチン



$C_{25}H_{38}O_5$:418.57

(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-Hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate [*79902-63-9*]

Simvastatin contains not less than 98.0% and not more than 101.0% of $C_{25}H_{38}O_5$, calculated on the dried basis.

It may contain a suitable antioxidant.

Description Simvastatin occurs as a white, crystalline powder. It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Simvastatin in acetonitrile (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*> and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Simvastatin 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 Simvastatin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Simvastatin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +285 - +300º (50 mg calculated on the dried basis, acetonitrile, 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1 g of Simvastatin in 10 mL of methanol: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 440 nm is not more than 0.10.

(2) Heavy metals <*1.07*>—To 1.0 g of Simvastatin add 2 mL of sulfuric acid, 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 0.5 mL of nitric acid, heat in the same manner as above, and ignite at 500 to 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution by

using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 30 mg of Simvastatin in 20 mL of a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0, (3:2), and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amounts of the peaks , having the relative retention times of about 0.45, about 0.80, about 2.42, and about 3.80 with respect to simvastatin are not more than 0.2%, respectively; the amount of the peak wsith a relative retention time of about 2.38 is not more than 0.3%; the amount of the peak with a relative retention time of about 0.60 is not more than 0.4%; and the amount of each peak other than simvastatin and other than the peaks mentioned above is not more than 0.1%. Furthermore, the total amount of the peaks other than simvastatin and other than the peak with relative retention time of about 0.60 with respect to simvastatin is not more than 1.0 %.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).

Mobile phase B: A solution of phosphoric acid in acetonitrile for liquid chromatography (1 in 1000).

Flowing of 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 (vol%)	Mobile phase B (vol%)
0 - 4.5	100	0
4.5 - 4.6	100 → 95	0 → 5
4.6 - 8.0	95 → 25	5 → 75
8.0 - 11.5	25	75

Flow rate: 3.0 mL per minute.

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

System suitability—

Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 0.5 mL of the sample solution, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, add a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0 (3:2), to make exactly 10 mL. Confirm that the peak area of simvastatin obtained from 5 μL of this solution is equivalent to 16 to 24% of the peak area of simvastatin in 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 5 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of simvastatin is not more than 1.0 %.

(4) Residual solvent—Being specified separately.

Loss on drying *<2.41>* Not more than 0.5% (1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Simvastatin and Simvastatin Reference Standard (previously determine the loss on drying *<2.41>* in the same manner as Simvastatin), dissolve each in a mixture of acetonitrile and 0.01 mol/L potassium dihydrogen phosphate TS, pH 4.0, (3:2) to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of simvastatin for each solution.

$$\text{Amount (mg) of simvastatin } (C_{25}H_{38}O_5) = W_S \times (A_T / A_S)$$

W_S: Amount (mg) of Simvastatin Reference Standard , calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 33 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile for liquid chromatography (1:1).

Flow rate: Adjust the flow rate so that the retention time of simvastatin is about 3 minutes.

System suitability—

System performance: Dissolve 3 mg of lovastatin in 2 mL of the standard solution. When the procedure is run with 5 μL of this solution under the above operating conditions, lovastatin and simvastatin 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 simvastatin is not more than 1.0 %.

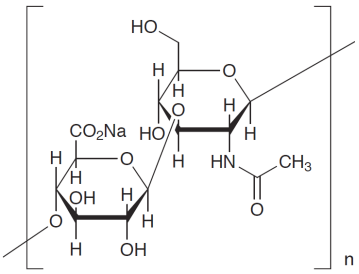
Containers and storage Containers—Tight containers.

Storage—Under nitrogen atmosphere.

Add the following:

Purified Sodium Hyaluronate

精製ヒアルロン酸ナトリウム



$(C_{14}H_{20}NNaO_{11})_n$
[*9067-32-7*]

Purified Sodium Hyaluronate is the sodium salt of glycosaminoglycans composed of disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine obtained from cockscomb or microorganisms.

It contains not less than 90.0% and not more than 105.5% of sodium hyaluronate $(C_{14}H_{20}NNaO_{11})_n$, calculated on the dried basis.

It is composed of an average molecular mass of the sodium salt of hyaluronic acid between 500,000 and 1,200,000 or between 1,500,000 and 3,900,000.

The average molecular mass of Purified Sodium Hyaluronate should be labeled.

Description Purified Sodium Hyaluronate occurs as white powder, granules or fibrous masses.

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

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Purified Sodium Hyaluronate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Purified Sodium Hyaluronate (1 in 1000) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Viscosity <*2.53*> Weigh accurately an amount of Purified Sodium Hyaluronate so that the downflowing time of its solution in 100 mL of 0.2 mol/L sodium chloride TS is 2.0 to 2.4 times longer than that of 0.2 mol/L sodium chloride TS, dissolve in 0.2 mol/L sodium chloride TS to make exactly 100 mL, and use this solution as the sample solution (1). Pipet 16 mL, 12 mL and 8 mL of the sample solution (1), to each add 0.2 mol/L sodium chloride TS to make exactly 20 mL, and use these solutions as the sample solutions (2), (3) and (4), respectively. Perform the test with the sample solutions (1), (2), (3) and (4) as directed under Method 1 at 30 ± 0.1ºC using a Ubbelohde-type viscometer in which the downflowing time for 0.2 mol/L sodium chloride is 200 to 300 seconds: the maximum viscosity calculated on the dried basis is between 10.0 and 19.5 dL/g or between 25.0 and 55.0 dL/g.

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Purified Sodium Hyaluronate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.20 g of Purified Sodium Hyaluronate in 15 mL of water, add 6 mL of dilute nitric acid, and heat on a water bath for 30 minutes. After cooling, add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.124%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Purified Sodium Hyaluronate 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) Residual solvent—Being specified separately.

(5) Protein—Weigh accurately about 20 mg of Purified Sodium Hyaluronate, calculated on the dried basis, dissolve in 1.0 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of bovine serum albumin, dissolve in dilute sodium hydroxide TS to make exactly 1000 mL, and use this solution as the standard solution. To 1.0 mL each of the sample solution and standard solution add 5.0 mL of alkaline copper TS (2), immediately stir, allow to stand at room temperature for 10 minutes, add 0.5 mL of diluted Folin's TS (1 in 2), immediately stir, and allow to stand at room temperature for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 1.0 mL of dilute sodium hydrochloride in the same manner, as the blank: the absorbance of the sample solution at 750 nm does not exceed the absorbance of the standard solution (not more than 0.05%).

(6) Nucleic acid— Determine the absorbance of a solution of 0.10 g Purified Sodium Hyaluronate in 50 mL of water as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank: the absorbance at 260 nm is not more than 0.02.

(7) Other acidic mucopolysaccharides—(In the case of chicken-derived samples) Dissolve 0.25 g of Purified Sodium Hyaluronate in 100 mL of water, and use this solution as the sample solution. Immerse a cellulose acetate membrane 6 cm in length in 0.2 mol/L pirydine-formic acid buffer solution, pH 3.0. Take out the membrane and remove excessive buffer solution using a filter paper. Place the membrane in an electrophoresis vessel saturated with 0.2 mol/L pyridine-formic acid buffer solution, pH 3.0, and run at 0.5 mA/cm for 1 minute. Apply 2 μL of the sample solution to the membrane in an area 1 cm in width at 1.5 cm from the anode. Carry out electrophoresis at 0.5 mA/cm for 1 hour. After the electrophoresis, stain the membrane by immersing it in Alcian blue staining solution for 10 to 20 minutes. After staining, decolorize sufficiently with diluted acetic acid (100) (3 in 100): no bands other than the principal band appears.

(8) Hemolytic streptococci—(In the case of microorganism-derived samples) Dissolve 0.5 g of Purified Sodium Hyalu-

ronate in sterile isotonic sodium chloride solution to make exactly 100 mL. Take 0.5 mL of this solution, apply to 2 blood agar plates, respectively, using a Conradi stick, and incubate at 37ºC for 48 hours: no hemolytic colonies appear, or if any, no streptococci are observed in the colony under a microscope.

(9) Hemolysis—(In the case of microorganism-derived samples) Dissolve 0.40 g of Purified Sodium Hyaluronate in sterile physiological saline to make exactly 100 mL. To 0.5 mL of this solution add 0.5 mL of 1% blood suspension, mix, allow to stand at 37ºC for 2 hours, and, if necessary, centrifuge at 3000 revolutions per minute for 10 minutes: the erythrocytes precipitate and the supernatant liquid is clear as in a blank determination performed in the same manner using 0.5 mL of isotonic sodium chloride as the blank and 0.5 mL of sterile purified water as the positive control.

Loss on drying *<2.41>* Not more than 15.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 60ºC, 5 hours).

Microbial limit *<4.05>* The allowable limits of the total aerobic microbial count and of the total combined yeasts/molds count for Purified Sodium Hyaluronate are 10^2 CFU/g and 10^1 CFU/g, respectively.

Average molecular mass **(1)** In the case of the labeled average molecular mass of between 500,000 and 1,200,000
Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 500,000 and 1,200,000. For $[\eta]$, use the maximum viscosity under Viscosity Determination.

$$\text{Average molecular mass} = ([\eta] \times 10^5 / 36)^{1/0.78}$$

(2) In the case of the labeled average molecular mass of between 1,500,000 and 3,900,000
Calculate the average molecular mass of Purified Sodium Hyaluronate according to the following equation: it is between 1,500,000 and 3,900,000. For $[\eta]$, use the maximum viscosity under Viscosity Determination.

$$\text{Average molecular mass} = ([\eta] \times 10^5 / 22.8)^{1/0.816}$$

Assay Weigh accurately about 50 mg of Purified Sodium Hyaluronate, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of D-Glucuronolactone Reference Standard, previously dried (under reduced pressure not exceeding 0.67 kPa, silica gel, 24 hours), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Pipet 1 mL each of the sample solution and standard solution, gently add into the 5.0 mL of sodium tetraborate-sulfuric acid TS, previously cooled in ice water, stir while cooling, heat in a water bath for 10 minutes, and cool in ice water. To each solution add 0.2 mL of carbazole TS, stir well, heat in a water bath for 15 minutes, and cool in ice water to room temperature. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at the wavelength of maximum absorption at 530 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, using a solution, prepared with 1 mL of water in the same manner, as the blank.

$$\text{Amount (mg) of sodium hyaluronate } [(C_{14}H_{20}NNaO_{11})_a] = W_S \times (A_T/A_S) \times 2.2786$$

W_S: Amount (mg) of D-Glucuronolactone Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant, at not exceeding 15ºC.

Sodium Iotalamate Injection

イオタラム酸ナトリウム注射液

Add the following next to Extractable volume:

Foreign insoluble matter *<6.06>* Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter *<6.07>* It meets the requirement.

Sterility *<4.06>* Perform the test according to the Direct method: it meets the requirement.

Sodium Valproate

バルプロ酸ナトリウム

Change the Origin/limits of content, Description, Identification and Purity to read:

Sodium Valproate, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{15}NaO_2$.

Description Sodium Valproate occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5) and in acetic acid (100). It is hygroscopic.

Identification **(1)** To 5mL of a solution of Sodium Valproate (1 in 20) add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

(2) Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of diethyl ether and 1 mL of 2 mol/L hydrochloric acid TS, and shake vigorously for 1 minute. Separate the diethyl ether layer, dehydrate with anhydrous sodium sulfate, and filter. Evaporate the solvent of the filtrate, determine the infrared spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Sodium Valproate (1 in 10) responds to the Qualitative Tests *<1.09>* for sodium salt.

Purity (1) Heavy metals <*1.07*>—Dissolve 2.0 g of Sodium Valproate in 44 mL of water, shake with 6 mL of dilute hydrochloric acid, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL with ammonia TS, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and methyl acetate (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of formic acid and methyl acetate (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of valproic acid from the sample solution is not more than the peak area of valproic acid from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with diethylene glycol adipate ester for gas chromatography and phosphoric acid at the ratios of 5% and 1%, respectively.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of valproic acid is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of valproic acid, beginning after the solvent peak.

System suitability—

System performance: To 2 mL of the sample solution and 8 μL of *n*-valerianic acid, add a mixture of formic acid and methyl acetate (1:1) to make 10 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, *n*-valerianic acid and valproic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: Pipet 2 mL of the standard solution and add a mixture of formic acid and methyl acetate (1:1) to make exactly 10 mL. When the test is repeated 6 times with 2 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of valproic acid is not more than 5.0%.

Add the following:

Sodium Valproate Syrup

バルプロ酸ナトリウムシロップ

Sodium Valproate Syrup contains not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Syrups, with Sodium Valproate.

Identification To a volume of Sodium Valproate Syrup, equivalent to 50 mg of Sodium Valproate according to the labeled amount, add water to make 10 mL. To 5 mL of this solution add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Microbial Limit Test <*4.05*> Acceptance criteria for the total aerobic microbial count and the total combined yeasts and moulds are 10^2 CFU and 10^1 CFU per mL of Sodium Valproate Syrup, respectively, with no Escherichia coli present.

Assay Pipet a volume of Sodium Valproate Syrup, equivalent to about 0.1 g of sodium valproate ($C_8H_{15}NaO_2$) and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sodium valproate for assay , previously dried at 105°C for 3 hours, dissolve in water to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of valproic acid to that of the internal standard.

$$\text{Amount (mg) of sodium valproate } (C_8H_{15}NaO_2) = W_S \times (Q_T / Q_S) \times 2$$

W_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50000)

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 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 sodium dihydrogenphosphate TS, pH 3.0 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of valproic acid 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 internal standard and valproic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Sodium Valproate Tablets

バルプロ酸ナトリウム錠

Sodium Valproate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sodium valproate ($C_8H_{15}NaO_2$: 166.19).

Method of preparation Prepare as directed under Tablets, with Sodium Valproate.

Identification To a quantity of powdered Sodium Valproate Tablets, equivalent to 0.5 g of Sodium Valproate according to the labeled amount, add 10 mL of water, shake well, and centrifuge. To 5 mL of the supernatant liquid add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20) and warm on a water bath: a purple precipitate is formed.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Sodium Valproate Tablets add 7 *V*/10 mL of the mobile phase, shake vigorously, add the mobile phase to make exactly *V* mL so that each mL contains about 1 mg of sodium valproate ($C_8H_{15}NaO_2$), and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, shake vigorously, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$)
$= W_S \times (Q_T / Q_S) \times (V / 100)$

W_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50000)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Sodium Valproate Tablets is not less than 85%.

Start the test with 1 tablet of Sodium Valproate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V′* mL so that each mL contains about 0.11 mg of sodium valproate ($C_8H_{15}NaO_2$), according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.11 mg of sodium valproate for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL of the solution, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of the valproic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of sodium valproate ($C_8H_{15}NaO_2$)
$= W_S \times (A_T / A_S) \times (V' / V) \times (1 / C) \times 180$

W_S: Amount (mg) of sodium valproate for assay
C: Labeled amount (mg) of sodium valproate ($C_8H_{15}NaO_2$) in 1 tablet.

Operating conditions—
Proceed as directed in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of valproic acid are not less than 3000 and not more than 2.0, respectively.

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 valproic acid is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Sodium Valproate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of sodium valproate ($C_8H_{15}NaO_2$), add about 160 mL of the mobile phase, shake well, add the mobile phase to make exactly 200 mL, and centrifuge. Filter the supernatant liquid, pipet 20 mL of the filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of sodium valproate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of valproic acid to that of the internal standard.

Amount (mg) of sodium valproate ($C_8H_{15}NaO_2$)
$= W_S \times (Q_T / Q_S) \times 2$

W_S: Amount (mg) of sodium valproate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (1 in 50000)
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 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 sodium dihydrogenphosphate TS, pH 3.0 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of

valproic acid 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 internal standard and valproic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valproic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Streptomycin Sulfate for Injection

注射用ストレプトマイシン硫酸塩

Streptomycin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Method of preparation Prepare as directed under Injections, with Streptomycin Sulfate.

Description Streptomycin Sulfate for Injection occurs as a white or light yellowish white masses or powder.

Identification Peform the test as directed in the Identification (2) under Streptomycin Sulfate.

Osmotic pressure ratio Being specified separately.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 10 mL of water is 5.0 to 7.0.

Purity Clarity and color of solution—Dissolve an amount of Streptomycin Sulfate for Injection, equivalent to 1.0 g (potency) of Streptomycin Sulfate according to the labeled amount, in 3 mL of water: The solution is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.50.

Loss on drying <*2.41*> Not more than 4.0 % (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 0.10 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass Variation Test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics<*4.02*> according to the following conditions.

(i) Test organisms, culture medium and standard solutions—Proceed as directed in the Assay under Streptomycin Sulfate.

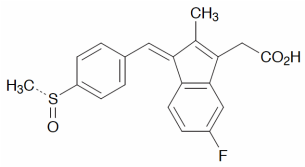
(ii) Sample solution Take 10 containers of Streptomycin Sulfate for Injection, and weigh accurately the mass of the contents. Weigh accurately an amount of the contents, equivalent to 1 g (potency) of Streptomycin Sulfate, and dissolve in water to make exactly 200 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make a 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—Hermetic containers.

Add the following:

Sulindac

スリンダク



and enantiomer

$C_{20}H_{17}FO_3S$:356.41

(1*Z*)-(5-Fluoro-2-methyl-1-{4-[(*RS*)-methylsulfinyl]benzylidene}-1*H*-inden-3-yl)acetic acid [*38194-50-2*]

Sulindac, when dried, contains not less than 99.0% and not more than 101.0% of $C_{20}H_{17}FO_3S$.

Description Sulindac occurs as a yellow, crystalline powder.

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

A solution of Sulindac in methanol (1 in 100) shows no optical rotation.

Melting point: about 184°C (with decomposition).

Identification (1) Dissolve 15 mg of Sulindac in 1000 mL of a solution of hydrochloric acid in methanol (1 in 120). Determine the absorption spectrum of this solution as directed under the Ultraviolet-visible Spectrophotometry <*2.24*> and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave-

lengths.

(2) Determine the infrared absorption spectrum of Sulindac as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Sulindac 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 <*1.11*>—Prepare the test solution with 1.0 g of Sulindac according to Method 3 and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.25 g of Sulindac in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL, 4 mL and 2 mL of this solution, to each add methanol to make exactly 10 mL, and use these solutions as the standard solution (1), standard solution (2) and standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 4μL each of the sample solution, standard solution (1), standard solution (2) and standard solution (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (97:3) to a distance of about 17 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 (1), and the total intensity of spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solution (1), standard solution (2) and standard solution (3).

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum not exceeding 0.7 kPa, 100°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.3 g of Sulindac, previously dried, dissolve in 50 mL of methanol and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

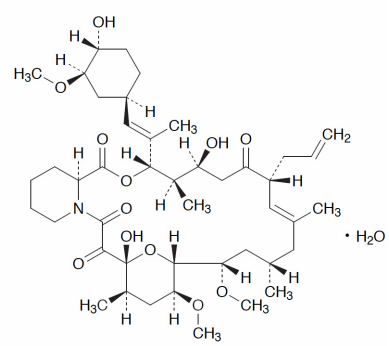
Each mL of 0.1 mol/L sodium hydroxide VS
= 35.64 mg of $C_{20}H_{17}FO_3S$

Containers and storage Containers—Tight containers.

Add the following:

Tacrolimus Hydrate

タクロリムス水和物



$C_{44}H_{69}NO_{12}\cdot H_2O$:822.03

(3*S*,4*R*,5*S*,8*R*,9*E*,12*S*,14*S*,15*R*,16*S*,18*R*,19*R*,26a*S*)-5,19-Dihydroxy-3-{(1*E*)-2-[(1*R*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl}-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(prop-2-en-1-yl)-15,19-epoxy-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-3*H*-pyrido[2,1-*c*][1,4]oxaazacyclotricosine-1,7,20,21(4*H*,23*H*)-tetrone monohydrate [*109581-93-3*]

Tacrolimus Hydrate contains not less than 98.0% and not more than 102.0% of tacrolimus ($C_{44}H_{69}NO_{12}$: 804.02), calculated on the anhydrous basis.

Description Tacrolimus Hydrate occurs as a white crystal or crystalline powder.

It is very soluble in methanol and in ethanol (99.5), freely soluble in *N*,*N*-dimethylformamide and in ethanol (95), and practically insoluble in water.

Identification **(1)** Dissolve 5 mg of Tacrolimus Hydrate in 1 mL of ethanol (95), add 1 mL of 1,3-dinitrobenzene TS and 1 mL of sodium hydroxide TS, and shake: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Tacrolimus Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tacrolimus Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -110 – -115º (0.2 g calculated on the anhydrous basis, *N*,*N*-dimethylformamide, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Tacrolimus Hydrate 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—Being specified separately.

(3) Residual solvent—Being specified separately.

Water <*2.48*> 1.9 – 2.5% (0.5 g, volumetric titration, direct

titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer Being specified separately.

Assay Weigh accurately about 25 mg each of Tacrolimus Hydrate and Tacrolimus Reference Standard (separately determine the water content <*2.48*> in the same manner as Tacrolimus Hydrate) and dissolve each in 15 mL of ethanol (99.5), to each add exactly 10 mL of the internal standard, add 25 mL of water, allow to stand for 6 hours, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tacrolimus to that of the internal standard.

Amount (mg) of tacrolimus ($C_{44}H_{69}NO_{12}$) = $W_S \times (Q_T / Q_S)$

W_S: Amount (mg) of Tacrolimus Reference Standard, calculated on the anhydrous basis.

Internal standard solution—A solution of heptyl parahydroxybenzoate in ethanol (99.5) (3 in 4000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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: Constant temperature of about 50°C.

Mobile phase: A mixture of water, 2-propanol for liquid chromatography and tetrahydrofuran for liquid chromatography (5:2:2).

Flow rate: Adjust the flow rate so that the retention time of tacrolimus 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, tacrolimus and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

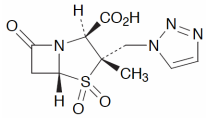
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tacrolimus to that of internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Tazobactam

タゾバクタム



$C_{10}H_{12}N_4O_5S$: 300.29

(2*S*,3*S*,5*R*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide [*89786-04-9*]

Tazobactam contains not less than 980 μg and not more than 1020 μg (potency) per 1 mg, calculated on the anhydrous basis. The potency of Tazobactam is expressed as mass (potency) of $C_{10}H_{12}N_4O_5S$.

Description Tazobactam occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in dimethylsulfoxide and in *N*, *N*-dimethylformamide, and slightly soluble in water, in methanol and in ethanol (99.5).

It dissolves in a solution of sodium hydrogen carbonate (3 in 100).

Identification **(1)** Determine the infrared absorption spectrum of Tazobactam as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tazobactam Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the spectrum of a solution of Tazobactam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 35) as directed under the Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.3 ppm, and double signals, B and C, at around δ 7.8 ppm and at around δ 8.1 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +162 – +167° (1 g calculated on the anhydrous basis, *N*, *N*-dimethylformamide, 100 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Tazobactam in 10 mL of sodium hydrogen carbonate (3 in 100) : the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> : the absorbance at 420 nm is not more than 0.14.

(2) Heavy metals <*1.07*> —Proceed with 1.0 g of Tazobactam 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).

(3) Related substances—This operation must be performed quickly. Dissolve 50 mg of Tazobactam 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 (1). Pipet 1 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 50 μL each of the sample solution, the standard solution (1) and the standard solution (2) as directed under the Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area form these solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.17 with respect to tazobactam, obtained from the sample solution is not larger than 4/5 times the peak area of tazobactam from the standard solution (1), the peak area other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect to tazobactam from the sample solution is not larger than the peak area of tazobactam from the standard solution (2), and the total area of the peaks other than the peak of tazobactam and the peak having the relative retention time of about 0.17 with respect of tazobactam from the sample solution is not larger than 2 times the peak area of tazobactam from the standard solution (2).

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 tazobactam.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution (1), and add the mobile phase to make exactly 20 mL. Confirm that the peak area of tazobactam obtained from 50 μL of this solution is equivalent to 3 to 7% of that of tazobactam from the standard (1).

System performance: When the procedure is run with 50 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 2000 and 0.8 -1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution (1) under the above operating conditions, the relative standard deviations of the peak area of tazobactam is not more than 1.0%.

(4) Residual solvent Being specified separately.

Water *<2.48>* Not more than 0.5% (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 *<2.44>* Not more than 0.1% (1 g).

Bacterial endotoxins *<4.01>* Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Tazobactam and Tazobactam Reference Standard, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add water 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 *<2.01>* according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak areas of tazobactam to that of the internal standard.

Amount [μg (potency)] of tazobactam ($C_{10}H_{12}N_4O_5S$)
$= W_S \times (Q_T / Q_S) \times 1000$

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

Internal standard solution—A solution of phenylalanine (1 in 400).

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 (10 μm in particle diameter).

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

Mobile phase: Disssolve 1.32 g of diammonium hydrogen phosphate in 750 mL of water, adjust the pH to 2.5 with phosphoric acid, add water to make 1000 mL, and add 25 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of tazobactam 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, the internal standard and tazobactam 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 tazobactam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Expiration date 24 months after preparation.

Teicoplanin

テイコプラニン

Change the Origin/limits of content to read:

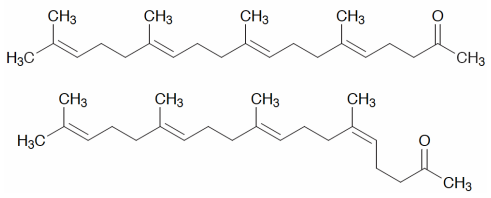
Teicoplanin is a mixture of glycopeptide substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.

It contains not less than 900 μg (potency) and not more than 1120 μg (potency) per 1 mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin ($C_{72\sim89}H_{68\sim99}Cl_2N_{8\sim9}O_{28\sim33}$).

Add the following:

Teprenone

テプレノン



$C_{23}H_{38}O$:330.55
(5*E*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one
(5*Z*,9*E*,13*E*)-6,10,14,18-Tetramethylnonadeca-5,9,13,17-tetraen-2-one
[*6809-52-5*]

Teprenone contains not less than 97.0% and not more than 101.0% of $C_{23}H_{38}O$.

Teprenone is comprised of mono-cis and all-trans isomers, with their ratio being about 2:3.

Description Teprenone occurs as a colorless to slightly yellowish clear oily liquid, with slight, characteristic odor.
It is miscible with ethanol (99.5), with ethyl acetate and with hexane.

It is practically insoluble in water.

It is oxidized by air, and gradually turns yellow.

Identification **(1)** To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 1 mL of a solution of phosphomolybdic acid *n*-hydrate in acetic acid (100) (1 in 100), heat in a water bath for 5 minutes, and continue heating with addition of 5 to 6 drops of sulfuric acid: blue to bluish green color develops.

(2) To 2 mL of a solution of Teprenone in ethanol (99.5) (1 in 100) add 2 mL of 2,4-dinitrophenylhydrazine TS, and shake: a yellow to orange-yellow precipitate is formed.

(3) Determine the infrared absorption spectrum of Teprenone as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Teprenone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <*2.45*> n_D^{20} : 1.485 – 1.491

Specific gravity <*2.56*> d_{20}^{20} : 0.882 – 0.890

Purity **(1)** Clarity and color of solution—To 1.0 mL of Teprenone add 9 mL of ethanol (99.5) and shake: the solution is clear, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*> is not more than 0.02.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Teprenone 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 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method and calculate the amounts of them by the area percentage method: the peak area of the di-cis isomer of teprenone, having the relative retention time of about 0.8 with respect to the all-trans isomer of teprenone, is not more than 0.5%, and each area of the peaks for the mono-cis and all-trans isomers of the teprenone and for those other than mentioned above is not more than 0.2 %. Furthermore, the total area of the peaks other than the mono-cis, all-trans and di-cis isomers of teprenone is not more than 1.0 %.

Operating conditions—

Detector, column, column temperature, carrier gas 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 for the all-trans isomer of teprenone beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add hexane 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 hexane to make exactly 10 mL. Confirm that the sum of the peak areas of the mono-cis and all-trans isomers of teprenone obtained from 3 μL of this solution is 7 to 13% of the peak areas of the mono-cis and all-trans isomers of teprenone from the solution for system suitability test.

System performance: When the procedure is run with 3 μL of the solution for system suitability test under the above operating conditions, the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between these peaks being not less than 1.1.

System repeatability: When the test is repeated 6 times with 3 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone is not more than 3.0%.

(4) Residual solvent—Being specified separately.

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer ratio Dissolve 30 mg of Teprenone in 6 mL of hexane, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having retention times of about 18 minutes, where A_a is the peak of the mono-cis isomer, having the shorter retention time, and A_b is the peak area of the all-trans isomer, having the longer retention time: A_a/A_b is 0.60 to 0.70.

Operating conditions—

Proceed as directed in the Assay.

System suitability—

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

Assay Weigh accurately about 50 mg each of Teprenone and Teprenone Reference Standard, dissolve each in exactly 5 mL of the internal standard solution, add ethyl acetate to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 3 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of teprenone (sum of the peak areas of mono-cis and all-trans isomers) to that of the internal standard.

$$\text{Amount (mg) of teprenone } (C_{23}H_{38}O) = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Teprenone Reference Standard

Internal standard solution—A solution of di-*n*-butyl phthalate in ethyl acetate (1 in 100)

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 2 m in length, packed with 149 to 177 μm siliceous earth for gas chromatography coated in 5% with polyethylene glycol 2-nitroterephthalate.

Column temperature: Constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of the peak of the all-trans isomer of teprenone, the larger of the two main peaks, having the retention time about 18 minutes, becomes 19 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and the mono-cis and all-trans isomers of teprenone are eluted in this order with the resolution between the mono-cis and all-trans isomers being not less than 1.1.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the sum of the peak areas of the mono-cis and all-trans isomers of teprenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight Containers.

Storage conditions—Under Nitrogen atmosphere at 2 to 8°C.

Testosterone Enanthate Injection

テストステロンエナント酸エステル注射液

Add the following next to Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> Perform the test according to Method 2: it meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Testosterone Propionate Injection

テストステロンプロピオン酸エステル注射液

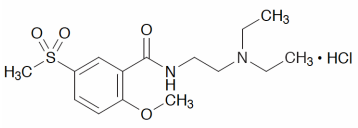
Add the following next to Foreign insoluble matter:

Insoluble particulate matter <*6.07*> Perform the test according to Method 2: it meets the requirement.

Add the following:

Tiapride Hydrochloride

チアプリド塩酸塩



$C_{15}H_{24}N_2O_4S{\cdot}HCl$: 364.89

N-[2-(Diethylamino)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide monohydrochloride

[*51012-33-0*]

Tiapride Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{15}H_{24}N_2O_4S{\cdot}HCl$.

Description Tiapride Hydrochloride occurs as a white to slightly yellowish white crystal or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in methanol, slightly soluble in ethanol (99.5) and very slightly soluble in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Tiapride Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, 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 Tiapride Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiapride Hydrochloride (1 in 20) responds to Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1 g of Tiapride Hydrochloride 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).

(2) Related substances—Dissolve 0.20 g of Tiapride Hydrochloride 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 1 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 Thin-layer Chromatography <*2.03*>. Spot rapidly10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of water, 1-butanol and acetic acid (100) (2:2:1) to a distance of about 10 cm, and air-dry, and then dry the plate at 80°C for 30 minutes. 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.

(3) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Tiapride Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.49 mg of $C_{15}H_{24}N_2O_4S$·HCl

Containers and storage Containers—Well-closed containers.

Add the following:

Tiapride Hydrochloride Tablets

チアプリド塩酸塩錠

Tiapride Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiapride ($C_{15}H_{24}N_2O_4S$: 328.43)

Method of preparation Prepare as directed under Tablets, with Tiapride Hydrochloride.

Identification To a quantity of powdered Tiapride Hydrochloride Tablets, equivalent to 10 mg of tiapride ($C_{15}H_{24}N_2O_4S$) according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 286 nm and 290 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiapride Hydrochloride Tablets add V/10 mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves until the tablet is disintegrated, and add 4V/10 mL of methanol. To this solution add exactly V/10 mL of the internal standard solution, shake for 30 minutes, and add methanol to make V mL so that each mL contains about 1 mg of tiapride ($C_{15}H_{24}N_2O_4S$). Centrifuge this solution for 10 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tiapride ($C_{15}H_{24}N_2O_4S$)
$= W_S \times (Q_T/Q_S) \times (V/100) \times 0.900$

W_S: Amount (mg) of tiapride hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 500)

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tiapride Hydrochloride Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tiapride ($C_{15}H_{24}N_2O_4S$), add about 10 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of methanol, add exactly 10 mL of the internal standard solution, shake for 30 minutes, and add methanol to make 100 mL. Centrifuge this solution and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.11 g of tiapride chloride for assay, previously dried at 105°C for 2 hours, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under liquid chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of Tiapride to that of the internal standard.

Amount (mg) of tiapride ($C_{15}H_{24}N_2O_4S$)
$= W_S \times (Q_T/Q_S) \times 0.900$

W_S: Amount (mg) of tiapride chloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (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: Constant temperature of about 25°C.

Mobile phase: Dissolve 11.2 g of sodium perchlorate in 800 mL of water, add 5 mL of diluted perchloric acid (17 in 2000). To 800 mL of this solution add 200 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, tiapride 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 ratio of the peak area of tiapride to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tolbutamide Tablets

トルブタミド錠

Add the following next to Identification:

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Change the Dissolution to read:

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of phosphate buffer solution, pH 7.4, as the dissolution medium, the dissolution rate in 30 minutes of Tolbutamide Tablets is not less than 80%.

Start the test with 1 tablet of Tolbutamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 10 μg of tolbutamide ($C_{12}H_{18}N_2O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tolbutamide Reference Standard, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 226 nm.

Dissolution rate (%) with respect to the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$)

$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

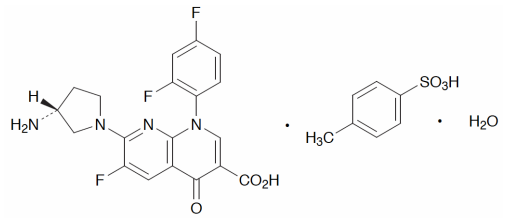
W_S: Amount (mg) of Tolbutamide Reference Standard

C: Labeled amount (mg) of tolbutamide ($C_{12}H_{18}N_2O_3S$) in 1 tablet

Add the following:

Tosufloxacin Tosilate Hydrate

トスフロキサシントシル酸塩水和物



and enantiomer

$C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$: 594.56

7-[(3*RS*)-3-Aminopyrrolidin-1-yl]-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid monotosylate monohydrate [*115964-29-9*, anhydride]

Tosufloxacin Tosilate Hydrate contains not less than 98.5% and not more than 101.0% of tosufloxacin tosilate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S$: 576.54), calculated on the anhydrous basis.

Description Tosufloxacin Tosilate Hydrate occurs as a white to pale yellowish white, crystalline powder.

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

A solution of Tosufloxacin Tosilate Hydrate in methanol (1 in 100) shows no optical rotation.

Melting point: about 254°C (with decomposition).

Identification (1) Tosufloxacin Tosilate Hydrate shows a light bluish-white fluorescence under ultraviolet light (main wavelength 254 nm).

(2) Proceed 10 mg of Tosufloxacin Tosilate Hydrate as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution responds to the Qualitative Tests <*1.09*> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Tosufloxacin Tosilate Hydrate in a mixture of methanol and sodium hydroxide TS (49:1) (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tosufloxacin Tosilate Hydrate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tosufloxacin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Tosufloxacin Tosilate Hydrate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Tosufloxacin Tosilate Hydrate in 40 mL of *N,N*-dimethylformamide, and add 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL (not more than 0.007%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tosufloxacin Tosilate Hydrate 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 <*1.11*>—Prepare the test solution with 1.0 g of Tosufloxacin Tosilate Hydrate according to Method 4, and perform the test under the condition of the ignition temperature being between 750 and 850°C, and add 10 mL of diluted hydrochloric acid to residue (not more than 2 ppm).

(4) Related substances—Dissolve10 mg of Tosufloxacin Tosilate Hydrate in 12 mL of mobile phase B, add water to make 25 mL, and use this solution as the sample solution. Pipet 5 mL of this solution, and add mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase A to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the area of each peak other than the peaks of tosylic acid and tosufloxacin from the sample solution is not more than 3/4 of the peak area of tosufloxacin from the standard solution, and the total area of the peaks other than those of tosylic acid and tosufloxacin from the sample solution is not larger than 2.5 times the peak area of tosufloxacin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Constant temperature of about 35ºC

Mobile phase A: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 143 mL of water, 40 mL of acetonitrile and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Mobile phase B: To 300 to 500 mL of water add slowly 100 mL of methanesulfonic acid under ice-cooling, add slowly 100 mL of triethylamine under ice-cooling too, and add water to make 1000 mL. To 10 mL of this solution add 100 mL of acetonitrile, 83 mL of water and 7 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Flowing of 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 (vol%)	Mobile phase B (vol%)
0 - 1	100	0
1 - 16	100 → 0	0 → 100
16 - 35	0	100

Flow rate: 0.5 mL per minute.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add mobile phase A to make exactly 20 mL. Confirm that the peak area of tosufloxacin obtained from 20 μL of this solution is equivalent to 18 to 32% of the peak area of tosufloxacin from 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 symmetry factor of the peak of tosufloxacin are not less than 10000 and not more than 1.5, 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 tosufloxacin is not more than 2.0%.

(5) Residual solvent Being specified separately.

Water <*2.48*> 2.5 – 3.5% (30 mg, coulometric titration).

Assay Weigh accurately about 30 mg each of Tosufloxacin Tosilate Hydrate and Tosufloxacin Tosilate Hydrate Reference Standard (separately determine the water content <*2.48*> in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve each in methanol to make exactly 100 mL. Pipet 20 mL each of these solutions, to each add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tosufloxacin to that of the internal standard.

Amount (mg) of tosufloxacin tosilate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S$)
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of Tosufloxacin Tosilate Reference Standard, calculated on the anhydrous basis.

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 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: Constant temperature of about 40ºC.

Mobile phase: To a mixture of 0.02 mol/L phosphate buffer solution, pH 3.5, and a solution of dibutylamine in methanol (1 in 2500) (3:1) add diluted phosphoric acid (1 in 10) to adjust the pH to 3.5.

Flow rate: Adjust the flow rate so that the retention time of tosufloxacin is about 20 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 tosufloxacin 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 ratio of the peak area of tosufloxacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Tosufloxacin Tosilate Tablets

トスフロキサシントシル酸塩錠

Tosufloxacin Tosilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tosuflozacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$: 594.56).

Method of preparation Prepare as directed under Tablets, with Tosufloxacin Tosilate Hydrate.

Identification To a quantity of powdered Tosufloxacin Tosilate Tablets, equivalent to 75 mg of Tosufloxacin Tosilate Hydrate according to the labeled amount, add 200 mL of a mixture of methanol and sodium hydroxide TS (49:1), shake well, and centrifuge. To 2 mL of the supernatant liquid add 100 mL of a mixture of methanol and sodium hydroxide TS (49:1). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 260 nm and 264 nm, between 341 nm and 345 nm, and between 356 nm and 360 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tosufloxacin Tosilate Tablets add V/10 mL of water and shake until the tablet is disintegrated. Add methanol to make exactly V mL so that each mL contains about 1.5 mg of tosufloxacin tosilate hydroxide ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$). Shake this solution for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$)

$= W_S \times (Q_T/Q_S) \times (V/20) \times 1.031$

W_S: Amount (mg) of Tosufloxacin Tosilate Reference Standard, calculated on the anhydrous basis.

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800)

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Tosufloxacin Tosilate Tablets is not less than 65%.

Start the test with 1 tablet of Tosufloxacin Tosilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly V' mL so that each mL contains about 17 μg of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of Tosufloxacin Tosilate Reference Standard (separately determine the water content <*2.48*> in the same manner as Tosufloxacin Tosilate Hydrate), and dissolve in *N*,*N*-dimethylformamide to make exactly 25 mL. Pipet 2 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the blank, and determine the absorbances, A_T and A_S, at 346 nm.

Dissolution rate (%) with respect to the labeled amount of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$)

$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 72 \times 1.031$

W_S: Amount (mg) of Tosufloxacin Tosilate Reference Standard, calculated on the anhydrous basis.

C: Labeled amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Tosufloxacin Tosilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$), add 10 mL of water and methanol to make exactly 100 mL, shake for 10 minutes, and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Tosufloxacin Tosilate Reference Standard (separately determine the water content <*2.48*> in the same manner as Tosufloxacin Tosilate Hydrate), add 2 mL of water, and dissolve in methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 4 mL of the internal standard solution and 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 standard solution as

directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tosufloxacin to that of the internal standard.

Amount (mg) of tosufloxacin tosilate hydrate ($C_{19}H_{15}F_3N_4O_3 \cdot C_7H_8O_3S \cdot H_2O$)
$= W_S \times (Q_T/Q_S) \times 5 \times 1.031$

W_S: Amount (mg) of Tosufloxacin Tosilate Reference Standard, calculated on the anhydrous basis.

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 800)

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Tosufloxacin Tosilate.

System suitability—

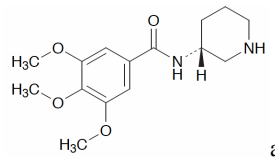
Proceed as directed in the system suitability in the Assay under Tosufloxacin Tosilate.

Containers and storage Containers—Well-closed containers.

Add the following:

Troxipide

トロキシピド



and enantiomer

$C_{15}H_{22}N_2O_4$: 294.35

3,4,5-Trimethoxy-*N*-[(3*RS*)-piperidin-3-yl]benzamide

[*30751-05-4*]

Troxipide, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{22}N_2O_4$.

Description Troxipide occurs as a white, crystalline powder.

It is freely soluble in acetate (100), soluble in methanol, sparingly soluble in ethanol (99.5) and slightly soluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Troxipide in 1 mol/L hydrochloric acid TS (1 in 5) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Troxipide in 0.1 mol/L hydrochloric acid TS (1 in 62500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Troxipide 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 Troxipide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Troxipide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 177 - 181°C

Purity (1) Chloride <*1.03*>—Dissolve 1.0 g of Troxipide in 30 mL of methanol, 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 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals <*1.07*>—Moisten 2.0 mg of Troxipide with 1 mL of sulfuric acid, and gently heat until charred. After cooling, add 2 mL of nitric acid, carefully heat until white fumes are no longer evolved, and perform the test according to Method 2. Prepare the control solution as follows: evaporate 1 mL of sulfuric acid, 2 mL of nitric acid and 2 mL of hydrochloric acid on a water bath and then on a sand bath to dryness, and moisten the residue with 3 drops of hydrochloric acid. Proceed in the same manner for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Troxipide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of this solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, water, hexane and ammonia water (28) (20:20:5:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): 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.

(4) Residual solvent—Being specified separately.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Troxipide, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.44 mg of $C_{15}H_{22}N_2O_4$

Containers and storage Containers—Tight containers.

Add the following:

Troxipide Fine Granules

トロキシピド細粒

Tiapride Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Powders, with Troxipide.

Identification To a quantity of Troxipide Fine Granules, equivalent to 20 mg of Troxipide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir, and filter. To 4 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units *<6.02>* Perform the test according to the following method: Tiapride Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

Take out the entire content of 1 pack of Troxipide Fine Granules, add 80 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide ($C_{15}H_{22}N_2O_4$). Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of troxipide } (C_{15}H_{22}N_2O_4) = W_S \times (Q_T/Q_S) \times (V/25)$$

W_S: Amount (mg) of Troxipide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Troxipide Fine Granules is not less than 85%.

Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.1 g of Troxipide ($C_{15}H_{22}N_2O_4$) according to the labeled amount, start the test, withdraw not less than 20 mL of the medium at the specified minute, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Troxipide Reference Standard, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and determine the absorbances, A_T and A_S, at 258 nm.

$$\text{Dissolution rate (\%) with respect to the labeled amount of troxipide } (C_{15}H_{22}N_2O_4) = (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 450$$

W_S: Amount (mg) of Troxipide standard for assay
W_T: Amount (mg) of Troxipide for Assay
C: Labeled amount (mg) of troxipide ($C_{15}H_{22}N_2O_4$) in 1 g

Particle Size *<6.03>* It meets the requirement.

Assay Weigh accurately an amount of Troxipide Fine Granules, equivalent to about 0.5 g of troxipide ($C_{15}H_{22}N_2O_4$), add 200 mL of 0.1 mol/L hydrochloric acid TS, stir for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of troxipide to that of the internal standard.

$$\text{Amount (mg) of troxipide } (C_{15}H_{22}N_2O_4) = W_S \times (Q_T/Q_S) \times 20$$

W_S: Amount (mg) of Troxipide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 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: Constant temperature of about 30ºC.

Mobile phase: To diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To 1500 mL of this solution add 100 mL of methanol and 50 mL of tetrahydronfuran.

Flow rate: Adjust the flow rate so that the retention time of troxipide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of standard solution under the above operating conditions, troxipide 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 ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Troxipide Tablets

トロキシピド錠

Troxipide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of troxipide ($C_{15}H_{22}N_2O_4$: 294.35).

Method of preparation Prepare as directed under Tablets, with Troxipide.

Identification Weigh accurately an amount of powdered Troxipide Tablets, equivalent to 0.1 g of Troxipide according to the labeled amount, add 250 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 4 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maximum between 256 nm and 260 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Troxipide Tablets add 90 mL of 0.1 mol/L hydrochloric acid TS, shake well to disintegrate, shake for another 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of troxipide ($C_{15}H_{22}N_2O_4$). Pipet 2 mL of the supernatant liquid, add exactly 3 mL of the internal standard solution, add water to make 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of troxipide } (C_{15}H_{22}N_2O_4) = W_S \times (Q_T/Q_S) \times (V/25)$$

W_S: Amount (mg) of Troxipide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Troxipide Tablets is not less than 70%.

Start the test with 1 tablet of Troxipide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg of troxipide ($C_{15}H_{22}N_2O_4$) according to the labeled amount, and use this solution as the sample solution. Separately weigh accurately about 20 mg of Troxipide Reference Standard, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 258 nm.

$$\text{Dissolution rate (\%) with respect to the labeled amount of troxipide } (C_{15}H_{22}N_2O_4) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S: Amount (mg) of Troxipide Reference Standard

C: Labeled amount (mg) of Troxipide ($C_{15}H_{22}N_2O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Troxipide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of troxipide ($C_{15}H_{22}N_2O_4$), add 150 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, and add 0.1 mol/L hydrochloric acid TS to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Troxipide Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 25 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of troxipide to that of the internal standard.

$$\text{Amount (mg) of troxipide } (C_{15}H_{22}N_2O_4) = W_S \times (Q_T/Q_S) \times 40$$

W_S: Amount (mg) of Troxipide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L hydrochloric acid TS (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 258 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: Constant temperature of about 30 °C.

Mobile phase: To 1500 mL of diluted phosphoric acid (1 in 500) add diethylamine to adjust the pH to 3.0. To this solution add 100 mL of methanol and 50 mL of tetrahydronfuran.

Flow rate: Adjust the flow rate so that the retention time of troxipide 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, troxipide 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 ratio of the peak area of troxipide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Ubenimex Capsules

ウベニメクスカプセル

Ubenimex Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$: 308.37).

Method of preparation Prepare as directed under Capsules, with Ubenimex.

Identification To a quantity of the contents of Ubenimex Capsules, equivalent to 25 mg of Ubenimex according to the labeled amount, add water to make 50 mL, shake well, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Ubenimex Capsules add 30 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 50 mL. Centrifuge this solution and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to about 3 mg of ubenimex ($C_{16}H_{24}N_2O_4$), add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 15 mL of this solution, add exactly 4 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ubenimex to that of the internal standard.

Amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4S$)
$= W_S \times (Q_T / Q_S) \times (1 / V) \times (15 / 2)$

W_S: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of water and acetonitrile (7:3) (1 in 2000)

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, ubenimex 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 2.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Ubenimex Capsules is not less than 70 %.

Start the test with 1 capsule of Ubenimex Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of water and acetonitrile (7:3) to make exactly V' mL so that each mL contains about 11 μg of ubenimex ($C_{16}H_{24}N_2O_4$) according to labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22mg of ubenimex for assay, previously dried in vacume at 80°C for 4 hours, and dissolve in a mixture of water and acetonitrile (7:3) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of water and acetonitrile (7:3) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ubenimex in each solution.

Dissolution rate (%) with respect to the labeled amount of ubenimex ($C_{16}H_{24}N_2O_4$)
$= W_S \times (A_T / A_S) \times (V' / V) \times (1 / C) \times 45$

W_S: Amount (mg) of ubenimex for assay
C: Labeled amount (mg) of ubenimex ($C_{16}H_{24}N_2O_4$) in 1 capsule

Operating conditions—

It meets test requirements directed in the assay.

System suitability—

System performance: When the procedure is run with conditions described above for 50 μL of the standard solution, the number of theoretical plates and symmetry factor for Ubenimex are not less than 3000 and not more than 2.0, respectively.

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 Ubenimex is not more than 2.0%.

Assay To 10 Ubenimex capsules add 140 mL of a mixture of water and acetonitrile (7:3), shake well for 30 minutes, and add a mixture of water and acetonitrile (7:3) to make exactly 200 mL. Centrifuge this solution, and filter. Discard the first 20 mL of the filtrate, pipet a volume of the subsequent filtrate, equivalent to about 7.5 mg of ubenimex ($C_{16}H_{24}N_2O_4$), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ubenimex for assay, previously dried at 80°C for 4 hours under reduced pressure, dissolve in a mixture of water and acetonitrile (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under liquid chromatography *<2.01>* according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ubenimex to that of the internal standard.

$$\text{Amount (mg) of ubenimex } (C_{16}H_{24}N_2O_4S) = W_S \times (Q_T / Q_S) \times (1 / 4)$$

W_S: Amount (mg) of ubenimex for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in mixture of water and acetonitrile (7:3) (1 in 2000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 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: Constant temperature of about 30 °C.

Mobile phase: A mixture of diluted phosphoric acid and acetonitrile for liquid chromatography (83:17) (1 in 100).

Flow rate: Adjust the flow rate so that the retention time of ubenimex 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, ubenimex 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ubenimex to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ursodeoxycholic Acid

ウルソデオキシコール酸

Change the Origin/limits of content, Description, Identification, and Purity and Assay to read:

Ursodeoxycholic Acid, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{40}O_4$.

Description Ursodeoxycholic Acid occurs as a white crystal or powder, with bitter taste.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Ursodeoxycholic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Sulfate *<1.14>*—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100), add water to make 200 mL, and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 40 mL of the sample solution 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 with 0.40 mL of 0.005 mol/L sulfuric acid by adding 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(2) Heavy metal *<1.07>*—Proceed with 1.0 g of Ursodeoxycholic Acid 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) Barium—To 2.0 g of Ursodeoxychollic Acid add 100 mL of water and 2 mL of hydrochloric acid, boil for 2 minutes, allow it to cool, filter, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is appeared.

(4) Related substances—Dissolve 0.10 g of Ursodeoxycholic Acid in 1 mL of methanol, add acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, and add acetone to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add acetone to make exactly 20 mL, and use these solutions as the standard solution (A) and standard solution (B), respectively. Separately, dissolve 50 mg of chenodeoxycholic acid for thin-layer chromatography in 5 mL of methanol, add acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution (1). Furthermore, dissolve 25 mg of lithocholic acid for thin-layer chromatography in 5 mL of methanol, and add acetone to make exactly 50 mL. Pipet 2 mL of this solution, and add acetone to make exactly 20 mL. Pipet 2 mL of this solution, add acetone to make exactly 10 mL, and use this solution as standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10μL each of the sample solution, stan-

dard solution (1), standard solution (2), standard solution (A) and standard solution (B) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately spray evenly the solution which was prepared by dissolving 5 g of phosphomolybdic acid *n*-hydrate in about 50 mL of ethanol (99.5), to which 5 mL of sulfuric acid is dropped in and add ethanol (99.5) to make 100 mL, and heat at 120°C for 3 to 5 minutes: the spots from the sample solution corresponding to the spots obtained from the standard solution (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), the spots other than the principal spot from the sample solution and other than those mentioned above are not intense than the spots obtained from the standard solution (B), and the total amount of the spots other than the principal spot from the sample solution and other than those mentioned above, which is calculated by the comparison with the spots obtained from the standard solutions (A) and (B), is not more than 0.25%.

Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Add the following:

Ursodeoxycholic Acid Granules

ウルソデオキシコール酸顆粒

Ursodeoxycholic Acid Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Granules, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic Acid Granules, equivalent to 20 mg of Ursodeoxycholic Acid according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and distil under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, immediately splay evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (99.5) (1 in 5), and heat at 120ºC for 3 to 5 minutes: the principle spot obtained from the sample solution and the spot from the standard solution show a blue color and the same R_f value.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the test solution, the dissolution rate in 15 minutes of Ursodeoxycholic Acid Granules is not less than 80 %.

Start the test by taking the exact amount that correspond to about 50 mg of ursodeoxycholic acid ($C_{24}H_{40}O_4$) according to labeled amount and withdraw not less than 20 mL of the medium at the specified time, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard first 10 mL of the filtrate, then use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of ursodeoxycholic acid for array, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL of the solution. Pipet 5 mL of this solution, add test solution to prepare exactly 20 mL of standard solution. Perform the test with 100 μL each of sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ursodeoxycholic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= (W_S / W_T) \times (A_T / A_S) \times (1 / C) \times 225$

W_S: Amount (mg) of ursodeoxycholic acid for assay
W_T: Amount (g) of Ursodeoxycholic Acid Glanules

C: Labeled amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$) in 1 g

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0 %.

Particle Size <*6.03*> It meets the requirement.

Assay Weigh accurately an amount of powdered Ursodeoxycholic Acid Granules, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$) according to labeled amount, add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g

of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in 20 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= W_S \times (Q_T / Q_S)$

W_S: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution— A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200000).

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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Constant temperature of about 40 °C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).

Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid 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, ursodeoxycholic acid 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 ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Ursodeoxycholic Acid Tablets

ウルソデオキシコール酸錠

Ursodeoxycholic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of Ursodeoxycholic Acid ($C_{24}H_{40}O_4$: 392.57).

Method of preparation Prepare as directed under Tablets, with Ursodeoxycholic Acid.

Identification To a quantity of powdered Ursodeoxycholic Acid Tablets, equivalent to 20 mg of Ursodeoxycholic Acid according to the labeled amount, add 10 mL of methanol, and shake for 20 minutes. Centrifuge this solution, pipet 4 mL of the supernatant liquid, and distil under reduced pressure. To the residue add 4 mL of acetone, disperse with ultrasonic waves, centrifuge, and use the remaining supernatant liquid as the sample solution. Separately, dissolve 10 mg of ursodeoxycholic acid in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of isooctane, ethanol (99.5), ethyl acetate and acetic acid (100) (10:6:3:1) to a distance of about 15 cm, and air-dry the plate. Dry the plate further at 120°C for 30 minutes, and immediately splay evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120ºC for 3 to 5 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show a blue color and the same R_f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Ursodeoxycholic Acid Tablets and add exactly V mL of the internal standard solution so that each mL contains about 5 mg of ursodeoxycholic acid ($C_{24}H_{40}O_4$), disperse it with ultrasonic waves, then agitate to mix for 10 more minutes and then centrifuge. Filter the clear supernatant solution through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution.

Proceed as directed in the operating conditions in the Assay

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= W_S \times (Q_T / Q_S) \times (V / 20)$

W_S: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg Ursodeoxycholic Acid tablet in 30 minutes and a 100-mg tablet in 45 minutes are not less than 80% and not less than 70%, respectively.

Start the test with one tablet of Ursodeoxycholic Acid Tablets, withdraw not less than 20 mL of the medium at the specified time after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard 10 mL of the first filtrate and pipet V mL of the subsequent filtrate. Add dissolution medium to make exactly V' mL so that each mL contains about 56 μg of ursodeoxycholic acid ($C_{24}H_{40}O_4$) according to the labeled amount, and use the solution as the sample solution. Separately weigh accurately about 22 mg of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of sample solution and standard solu-

tion as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ursodeoxycholic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= W_S \times (A_T / A_S) + (V'/ V) + (1 / C) \times 225$

W_S: Amount (mg) of ursodeoxycholic acid for assay
C: Labeled amount (mg) of ursodeoxycholic acid in 1 tablet ($C_{24}H_{40}O_4$)

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating condition, the number of theoretical plates and symmetry factor of the peak of ursodeoxycholic acid are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ursodeoxycholic acid is not more than 2.0 %.

Assay Weigh accurately the mass of not less than 20 Ursodeoxycholic Acid Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of ursodeoxycholic acid ($C_{24}H_{40}O_4$), add exactly 20 mL of the internal standard solution, shake for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 0.1 g of ursodeoxycholic acid for assay, previously dried at 105°C for 2 hours, dissolve in exactly 20 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ursodeoxycholic acid to that of the internal standard.

Amount (mg) of ursodeoxycholic acid ($C_{24}H_{40}O_4$)
$= W_S \times (Q_T / Q_S)$

W_S: Amount (mg) of ursodeoxycholic acid for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (4 in 5) (7 in 200000).
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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: Constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile for liquid chromatography (11:9).
Flow rate: Adjust the flow rate so that the retention time of ursodeoxycholic acid is about 6 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, ursodeoxycholic acid 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 ratio of the peak area of ursodeoxycholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Vasopressin Injection

バソプレシン注射液

Add the following next to Purity:

Bacterial endotoxins <*4.01*> Less than 15 EU / Vasopressin unit

Add the following next to Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.
Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Warfarin Potassium Tablets

ワルファリンカリウム錠

Add the following next to Uniformity of dosage units:

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates of 0.5-mg, 1-mg and 2-mg tablets in 15 minutes and of 5-mg tablets in 30 minutes are not less than 80%.

Start the test with 1 tablet of Warfarin Potassium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of warfarin potassium ($C_{19}H_{15}KO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Warfarin Potassium Reference Standard, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use

this solution as the standard solution. Perform the test with 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of warfarin from each solution.

Dissolution rate (%) with respect to the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$)
$= W_S \times (A_T / A_S) \times (V' / V) \times (1 / C) \times (9 / 4)$

W_S: Amount (mg) of Warfarin Potassium Reference Standard
C: Labeled amount (mg) of warfarin potassium ($C_{19}H_{15}KO_4$) in 1 tablet

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 35°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (700:300:1).

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

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of warfarin are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Wheat Starch

コムギデンプン

Change the Origin/limits of content and the Identification (1) to read:

Wheat Starch consists of the starch granules obtained from caryopsis of wheat, *Triticum aestivum* Linné (*Gramineae*).

Identification (1) Examine under a microscope <5.01> using a mixture of water and glycerinol (1:1), Wheat Starch presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually 10-60 μm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2-10 μm in diameter. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

Add the following next to item (3) in the section on Purity.

Purity ◆**(4)** Foreign matter—Under a microscope <5.01>, Wheat Starch does not contain starch granules of any other origin.◆

Zinc Sulfate Hydrate

硫酸亜鉛水和物

Change the Description and Identification to read:

Description Zinc Sulfate Hydrate occurs as colorless crystals or white crystalline powder.

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

It effloresces in dry air.

Identification (1) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for zinc salt.

(2) A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

Add the following next to the Identification:

pH <*2.54*> Dissolve 1.0 g of Zinc Sulfate Hydrate in 20 mL of water: the pH of the solution is between 4.4 and 6.0.

Change the Purity (1) to read:

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Zinc Sulfate Hydrate in 5 mL of water: the solution is clear and colorless.

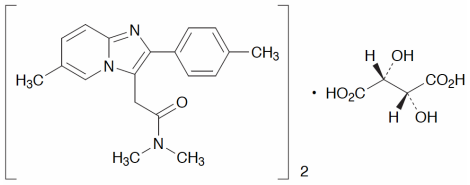
Add the following next to the Purity:

Loss on drying <*2.41*> Not less than 35.5% and not more than 38.5% (1 g, 105°C, 3 hours).

Add the following:

Zolpidem Tartrate

ゾルピデム酒石酸塩



$(C_{19}H_{21}N_3O)_2{\cdot}C_4H_6O_6$:764.87

N,*N*,6–Trimethyl–2–(4–methylphenyl)imidazo[1,2–*a*]pyridine–3-acetamide hemi–(2*R*,3*R*)–tartrate [*99294–93–6*]

Zolpidem Tartrate contains not less than 98.5% and not more than 101.0% of zolpidem tartrate $[(C_{19}H_{21}N_3O)_2{\cdot}C_4H_6O_6)]$, calculated on the anhydrous basis.

Description Zolpidem Tartrate occurs as a white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in *N*,*N*-dimethylformamide and in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5) and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It gradually changes to yellow in color on exposure to light.

Optical rotation $[\alpha]_D^{20}$: About +1.8° (1 g, *N*,*N*-dimethylformamide, 20 mL, 100 mm).

Identification **(1)** Dissolve 50 mg of Zolpidem Tartrate in 5 mL of acetic acid (100) and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Zolpidem Tartrate in 0.1 mol/L hydrochloric acid TS (1 in 100000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, 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 Zolpidem Tartrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Zolpidem Tartrate in methanol (1 in 10) responds to the Qualitative Tests *<1.09>* (3) for tartrate.

Purity **(1)** Heavy metals *<1.07>*—Proceed with 2.0 g of Zolpidem Tartrate 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—Dissolve 10 mg of Zolpidem Tartrate in 20 mL of methanol and use this solution as the sample solution. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peak other than the peak of zolpidem from the sample solution is not more than the peak area of zolpidem from the standard solution.

Operating conditions—

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

Column: A stainless steel tube 4.6 mm in inside diameter and 7.5 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 4.9 g of phosphoric acid add 1000 mL of water, and adjust the pH to 5.5 with triethylamine. To 11 volumes of this solution add 5 volumes of methanol and 4 volumes of acetonitrile.

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

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

System suitability—

System performance: Dissolve 10 mg each of Zolpidem Tartrate and benzyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 5 μL of this solution under the above operating conditions, zolpidem and benzyl parahydroxybenzoate 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 peak area of zolpidem is not more than 5.0%.

(3) Residual solvent—Being specified separately.

Water *<2.48>* Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Zolpidem Tartrate, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) and titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.24 mg of $(C_{19}H_{21}N_3O)_2{\cdot}C_4H_6O_6$

Containers and storage Containers—Tight containers

Storage conditions—Light-resistant

Crude Drugs

Apricot Kernel

キョウニン

Change the Origin to read:

Apricot Kernel is the seed of *Prunus armeniaca* Linné or *Prunus armeniaca* Linné var. *ansu* Maximowicz (*Rosaceae*).

It contains not less than 2.0% of amygdalin, calculated on the basis of dried material.

Add the following next to the Purity:

Loss on drying <*5.01*> Not more than 7.0% (6 hours).

Component determination Weigh accurately 0.5 g of ground Apricot Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = W_S \times (A_T/A_S) \times 2$$

W_S : Amount (mg) of amygdalin for component determination

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 octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin 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, the number of theoretical plates and the symmetry factor of the peak of amygdalin 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 amygdalin is not more than 1.5%.

Astragalus Root

オウギ

Add the following next to Description:

Identification Put 1 g of pulverized Astragalus Root in a glass-stoppered centrifuge tube, add 5 mL of potassium hydroxide TS and 5 mL acetonitrile, and stop the vial tightly. After shaking this for 10 minutes, centrifuge, and use the upper layer as the sample solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and R_f value with the brownish yellow fluorescent spot from the standard solution.

Bear Bile

ユウタン

Change the Identification to read:

Identification To 0.1 g of pulverized Bear Bile, add 5 mL of methanol, heat on a water bath for 10 minutes, centrifuge after cooling, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 5 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetic acid (100), toluene and water (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the spot from the standard solution.

Add the following next to the Identification:

Purity Other animal biles—Use the sample solution obtained in the Identification as the sample solution. Separately, dissolve 10 mg of sodium glycocholate for thin-layer chromatography and 20 mg of powdered porcine bile for thin-layer chromatography in 5 mL of methanol, and use these solutions as the standard solution (1) and standard solution (2) respectively. Perform the test with these solutions as directed in the Identification: Spots from the sample solution correspond to neither the spot of glycocholic acid from the standard solution (1) nor the gray-brown to black spot of powdered porcine bile at around R_f 0.3 from the standard solution (2).

Bupleurum Root

サイコ

Change the Identification (2) to read:

Identification (2) To 1.0 g of the pulverized Bupleurum Root, add 10 mL of methanol, and boil gently under a reflux condenser on a water bath for 15 minutes. After cooling, centrifuge, and use the filtrate as the sample solution. Separately, dissolve 1 mg of saikosaponin a 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 Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel 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. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the gray-brown spot from the standard solution, accompanied by the adjacent yellow-red spot above.

Calumba

コロンボ

Change the Purity to read:

Purity (1) Heavy metals *<1.07>* Proceed with 3.0 g of pulverized Calumba according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic *<1.11>* Prepare the test solution with 0.40 g of pulverized Calumba according to Method 4, and perform the test (not more than 5 ppm).

Powdered Calumba

コロンボ末

Change the Purity to read:

Purity (1) Heavy metals *<1.07>* Proceed with 3.0 g of Powdered Calumba according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic *<1.11>* Prepare the test solution with 0.40 g of Powdered Calumba according to Method 4, and perform the test (not more than 5 ppm).

Cardamon

ショウズク

Change the another title in Japanese of the monograph as follows:

小豆寇

小豆蔻

Cornus Fruit

サンシュユ

Change the Origin to read:

Cornus Fruit is the pulp of the pseudocarp of *Cornus officinalis* Siebold et Zuccarini (*Cornaceae*).

It contains not less than 0.4% of loganin, calculated on the basis of dried material.

Add the following next to Extract Content:

Component determination Weigh accurately about 1 g of fine cuttings of Cornus Fruit (separately determine the loss on drying *<5.01>*), put in a glass-stoppered centrifuge tube, suspend in 30 mL of diluted methanol (1 in 2), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 30 mL of diluted methanol (1 in 2), and repeat the above process twice more. Combine all the extracts, 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 loganin for component determination, previously dried in a desiccator (silica gel) for 24 hours, dissolve in 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = W_S \times (A_T/A_S)$$

W_S **:** Amount (mg) of loganin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm)

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

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

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: Adjust the flow rate so that the retention time of loganin is about 25 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 factor of the peak of loganin 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 loganin is not more than 1.5%.

Daiokanzoto Extract

大黄甘草湯エキス

Change the Origin to read:

Daiokanzoto Extract contains not less than 3.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) and not less than 9 mg and not more than 27 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 18 mg and not more than 54 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per the extract prepared as directed in the Method of preparation.

Eleutherococcus Senticosus Rhizome

シゴカ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*> Proceed with 3.0 g of pulverized Eleutherococcus Senticosus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.40 g of pulverized Eleutherococcus Senticosus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Add the following:

Goshajinkigan Extract

牛車腎気丸エキス

Goshajinkigan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.2 mg (for preparation prescribed Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) per the extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 5 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Poria Sclerotium, 3 g of Moutan Bark, 1 g of Cinnamon Bark, 1 g of Powdered Processed Aconite Root 1 or Powdered Processed Aconite Root 2 of "Powdered Processed Aconite Root", 3 g of Achyranthes Root and 3 g of Plantago Seed.

Description Goshajinkigan Extract occurs as brown to black-brown powder or viscous extract. It has slightly a characteristic odor and a slightly acid taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at around R_f 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of 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 (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Alisol A for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 2μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100)(10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Alisma Rhizome).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 2μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde 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 Thin-layer Chromatography *<2.03>*. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde 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 Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (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 spot among several spots from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 10 μL of 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 Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Powdered Processed Aconite Root).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.3 g of pulverized Plantago Seed for thin-layer chromatography, add 1 mL of methanol, heat on a water bath for 3 minutes, centrifuge after cooling, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel 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. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value (around 0.3) with the deep blue spot from the standard solution (Plantago Seed).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 2 g of Achyranthes Root for thin-layer chromatography, add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL

each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value (around 0.4) with the dark red spot from the standard solution (Achyranthes Root).

Purity **(1)** Heavy metals *<1.07>* Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic *<1.11>* Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)— Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

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 phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying *<2.41>* The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash *<5.01>* Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of loganin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of loganin.

$$\text{Amount (mg) of loganin} = W_S \times (A_T / A_S) \times (1/2)$$

W_S: Amount (mg) of loganin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 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 water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 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 symmetry factor of the peak of loganin 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 loganin is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry

extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water), and 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of peoniflorin.

$$\text{Amount (mg) of peoniflorin } (C_{23}H_{28}O_{11}) = W_S \times (A_T / A_S) \times (1/2)$$

W_S: Amount (mg) of Peoniflorin 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: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin 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 peoniflorin 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 peoniflorin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for component determination as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

$$\text{Amount (mg) of benzoylmesaconine hydrochloride} = C_{SM} \times (A_{TM} / A_{SM}) \times 10$$

$$\text{Amount (mg) of benzoylhypaconine hydrochloride} = C_{SH} \times (A_{TH} / A_{SH}) \times 10$$

$$\text{Amount (mg) of 14-anisoylaconine hydrochloride} = C_{SA} \times (A_{TA} / A_{SA}) \times 10$$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylhypaconine and benzoylmesaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Container and storage Containers—Tight containers.

Add the following:

Hachimijiogan Extract

八味地黄丸エキス

Hachimijiogan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg (for preparation prescribed 3 g of Moutan Bark) or not less than 5 mg and not more than 15 mg (for preparation prescribed 2.5 g of Moutan Bark) of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride), or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) per the extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 5 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Poria Sclerotium, 3 g of Moutan Bark, 1 g of Cinnamon Bark, and 1 g of Processed Aconit Root 1 of "Processed Aconit Root" or Powdered Processed Aconit Root 1 or Powdered Processed Aconit Root 2 of "Powdered Processed Aconit Root", or with 6 g of Rehmannia Root, 3 g of Cornus Fruit, 3 g of Dioscorea Rhizome, 3 g of Alisma Rhizome, 3 g of Poria Sclerotium, 2.5 g of Moutan Bark, 1 g of Cinnamon Bark, and 0.5 g of Powdered Processed Aconit Root 1 of "Powdered Processed Aconit Root".

Description Hachimijiogan Extract occurs as grayish brown to black-brown powder or viscous extract. It has a characteristic odor and a slightly bitter and acid taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, heat at 105ºC for 5 minutes, and allow to cool; a dark-green spot is observed at around R_f 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL of the sample solution and 2 μL of 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 (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105ºC for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Alisol A for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 2μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105ºC for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Alisma Rhizome).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105ºC for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde 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 Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde 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 Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (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 spot among several spots from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of 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 Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate : one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

Purity **(1)** Heavy metals <*1.07*> Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)— Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet exactly 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

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 40ºC.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5 %.

Loss on drying <*2.41*> The dry extract: Not more than 8.5 % (1 g, 105ºC, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105ºC, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Loganin—Weigh accurately about 0.5 g of the dry

extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of loganin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_s, of loganin.

$$\text{Amount (mg) of loganin} = W_S \times (A_T / A_s) \times (1/2)$$

W_S: Amount (mg) of loganin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 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 50ºC.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 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 symmetry factor of the peak of loganin 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 loganin is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water), and 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_s, of peoniflorin.

$$\text{Amount (mg) of peoniflorin } (C_{23}H_{28}O_{11}) = W_S \times (A_T / A_S) \times (1/2)$$

W_S: Amount (mg) of Peoniflorin 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 20ºC.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin 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 peoniflorin 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 peoniflorin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for component determination as directed under Liquid Chromatography *<2.01>* according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

$$\text{Amount (mg) of benzoylmesaconine hydrochloride} = C_{SM} \times (A_{TM} / A_{SM}) \times 10$$

$$\text{Amount (mg) of benzoylhypaconine hydrochloride} = C_{SH} \times (A_{TH} / A_{SH}) \times 10$$

$$\text{Amount (mg) of 14-anisoylaconine hydrochloride} = C_{SA} \times (A_{TA} / A_{SA}) \times 10$$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for component determination in aconitum

monoester alkaloids standard solution TS for component determination

C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylhypaconine and benzoylmesaconine; 254 nm for 14-anisoylaconine).

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 40ºC.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Container and storage Containers—Tight containers.

Hochuekkito Extract

補中益気湯エキス

Change the Origin to read:

Hochuekkito Extract contains not less than 16 mg and not more than 64 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikoponin b_2, and not less than12 mg and not more than 36 mg of grycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per the extract prepared as directed in the Method of preparation.

Japanese Valerian

カノコソウ

Change the Purity to read:

Purity (1) Heavy metals <*1.07*> Proceed with 3.0 g of pulverized Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.40 g of pulverized Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

Powdered Japanese Valerian

カノコソウ末

Change the Purity to read:

Purity (1) Heavy metals <*1.07*> Proceed with 3.0 g of Powdered Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.40 g of Powdered Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

Kakkonto Extract

葛根湯エキス

Change the Origin to read:

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephendra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephendra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 14 mg and not more than 56 mg (for preparation prescribed 2 g of Peony Root) of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46) or not less than 21 mg and not more than 84 mg (for preparation prescribed 3 g of Peony Root), and not less than 19 mg and not more than 57 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per the extract prepared as directed in the Method of preparation.

Lithospermum Root

シコン

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*> Proceed with 3.0 g of pulverized Lithospermum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.40 g of pulverized Lithospermum Root according to Method 4, and perform the test (not more than 5 ppm).

Add the following:

Longan Aril

Longan Arillus

リュウガンニク

Longan Aril is the aril of *Euphoria longana* Lamarck (*Sapindaceae*).

Description Depressed ellipsoidal aril, 1 - 2 cm in length, about 1 cm in width; yellowish red-brown to blackish brown; soft in texture and mucous; when immersed in water, bell-shaped, with the tip split in several parts.

Odor, characteristic; taste, sweet.

Under a microscope <*5.01*>, a transverse section reveals the outmost layer composed of a single-layered epidermis, beneath this observed parenchyma consisting of depressed parenchyma cells; the innermost layer composed of slightly thick-walled epidermis; parenchyma contains red-brown to brown contents as well as solitary crystals, amorphous crystals and sand crystals of calcium oxalate.

Identification To 1 g of coarse cuttings of Longan Aril, add 10 mL of water, shake thoroughly, and filter. To 3 mL of the filtrate, add 3 mL of Fehling solution, and heat on a water bath: a red precipitate is produced.

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: Not less than 75.0%.

Longgu

リュウコツ

Change (2) of the Identification and (1) of the Purity to read:

Identification **(2)** The turbid solution obtained in (1) has a characteristic odor. Filtrate this solution and neutralize with ammonia TS: this solution responds to the Qualitative Tests <*1.09*> (1), (2) and (3) for calcium salt.

Purity **(1)** Heavy metals <*1.07*> To 2.0 g of pulverized Longgu, add 5 mL of water, shake, add gradually 6 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate, add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test with this solution as the test solution. Separately, evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL, and use this solution as the control solution (not more than 20 ppm).

When being shown as extracts, infusions and decoctions on the label, the procedure and the limit are as follows.

To 20.0 g of pulverized Longgu, add 80 mL of water, shake occasionally on a water bath, heat to make about 40 mL, allow to cool, and filter. Proceed with this solution according to Method 3, and perform the test. To the control solution, add 1.0 mL of Standard Lead Solution (not more than 0.5 ppm).

Nuphar Rhizome

センコツ

Change the Purity to read:

Purity **(1)** Petiole—The amount of the petioles contained in Nuphar Rhizome does not exceed 3.0%.

(2) Heavy metals <*1.07*> Proceed with 3.0 g of pulverized Nuphar Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*> Prepare the test solution with 0.4 g of pulverized Nuphar Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*> The amount of foreign matter other than the petioles is not more than 1.0%.

Add the following:

Nutmeg

Myristicae Semen

ニクズク

Nutmeg is the seed of *Myristica fragrans* Houttuyn (*Myristicaceae*), usually from which the seed coat has been removed.

Description Ovoid-globose to ellipsoidal seeds, 1.5 - 3.0 cm in length, 1.3 - 2.0 cm in diameter; externally grayish brown, with wide and shallow longitudinal furrows and fine wrinkles; usually, grayish white to grayish yellow and slightly protruding hilum at one end, grayish brown to dark brown and slightly concave chalaza at the other end; cross section has a marble-like appearance with the dark brown thin perisperm extending ir-

regularly into the light yellowish white to light brown endosperm.

Odor, characteristic and strong; taste, acrid and slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals perisperm composed of outer and inner layers, the outer layer composed of parenchyma containing dark red-brown contents and the inner layer composed of parenchyma containing red-brown contents with a number of large oil cells and scattered vascular bundles; in parenchyma cells of endosperm, simple or compound starch grains and aleurone grains observed.

Identification To 1 g of pulverized Nutmeg, add 5 mL of methanol, allow to stand for 10 minutes with occasional shaking, filtrate, and use the filtrate as the sample solution. Separately, dissolve 2 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the red-purple spot from the standard solution.

Loss on drying <*5.01*> Not more than 16.0% (6 hours).

Total ash <*5.01*> Not more than 2.5%.

Essential oil content <*5.01*> When the test is performed with 10.0 g of pulverized Nutmeg, the essential oil content is not less than 0.5 mL.

Peach Kernel

トウニン

Change the Origin to read:

Peach Kernel is the seed of *Prunus persica* Batsch or *Prunus persica* Batsch var. *davidiana* Maximowicz (*Rosaceae*).

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

Add the following next to the Purity:

Loss on drying <*5.01*> Not more than 8.0% (6 hours).

Component determination Weigh accurately 0.5 g of ground Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = W_S \times (A_T/A_S) \times 2$$

W_S **:** Amount (mg) of amygdalin for component determination

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 octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin 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, the number of theoretical plates and the symmetry factor of the peak of amygdalin 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 amygdalin is not more than 1.5%.

Powdered Peach Kernel

トウニン末

Change the Origin to read:

Powdered Peach Kernel is the powder of Peach Kernel.

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

Add the following next to the Acid-insoluble ash:

Component determination Weigh accurately 0.5 g of Powdered Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) 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 standard solution as directed under

Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = W_S \times (A_T/A_S) \times 2$$

W_S : Amount (mg) of amygdalin for component determination

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 octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin 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, the number of theoretical plates and the symmetry factor of the peak of amygdalin 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 amygdalin is not more than 1.5%.

Perilla Herb

ソヨウ

Delete the Essential oil content and change the Origin and the Identification to read:

Perilla Herb is the leaves and the tips of branches of *Perilla frutescens* Britton var. *acuta* Kudo or *Perilla frutescens* Britton var. *crispa* Decaisne (*Labiatae*).

It contains not less than 0.08% of perillaldehyde, calculated on the basis of dried material.

Identification To 0.6 g of pulverized Perilla Herb, add 10 mL of diethyl ether, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the red-purple spot from the standard solution.

Add the following next to the Acid-insoluble ash:

Component determination Weigh accurately about 0.2 g of freshly prepared pulverized Perilla Herb, put in a glass-stoppered centrifuge tube, add 20 mL of methanol, shake for 10 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 20 mL of methanol, and proceed in the same manner. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of perillaldehyde for component determination, and dissolve in methanol to make exactly 100 mL. Weigh accurately 10 mL of this solution, add methanol 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of perillaldehyde in each solution.

$$\text{Amount (mg) of perillaldehyde} = W_S \times (A_T / A_S) \times (1/20)$$

W_S: Amount (mg) of perillaldehyde for component determination

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 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: 1.0 mL per minute

System suitability—

System performance: Dissolve 1 mg of (*E*)-asarone in the standard solution to make exactly 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, perillaldehyde and (*E*)-asarone 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 perillaldehyde is not more than 1.5%.

Add the following:

Pogostemon Herb

Pogostemoni Herba

カッコウ

Pogostemon Herb is the terrestrial part of *Pogostemon cablin* Bentham (*Labiatae*).

Description Stems with opposite leaves, leaves wrinkled and shriveled. When smoothed by immersion in water, leaves are obovate to ovate-oblong, 2.5-10 cm in length, 2.5-7 cm in width, with obtusely serrate margins and petioles at the cuneate bases; the upper surface of leaves dark brown, the lower surface gray-

ish brown, both sides covered densely with hairs. Stems are square, solid, grayish green, covered with grayish to yellowish white hairs; the pith broad, whitish, spongy. Under a magnifying glass, leaf reveales hairs, glandular hairs and glandular scales.

Odor, distinct; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of petiole reveals central portion of the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center divided into two groups. Under a microscope <5.01>, a transverse section of the midvein of lamina reveals the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center arranged in fan-shape. Under a microscope <*5.01*>, a transverse section of stem reveals several-cells-layered collenchyma beneath epidermis, occasionally with cork layer developed; beneath cortex, collateral vascular bundles arranged in a circle, phloem fibers in groups observed at the outer portion of phloem; oil droplets observed in parenchymat cells of cortex,, needle, solitary or columnar crystals of calcium oxalate in parenchyma cells of pith.

Identification To 0.5 g of pulverized Pogostemon Herb, add 5 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. 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 acetone (9:1) 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; a red spot appears at around R_f 0.4.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 13.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> When the test is performed with 50.0 g of pulverized Pogostemon Herb in a flask with 1 mL of silicon resin added, the essential oil content is not less than 0.3 mL.

Polygonatum Rhizome

オウセイ

Add the following next to Identification:

Purity (1) Heavy metals <*1.07*> Proceed with 3.0 g of pulverized Polygonutum Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.40 g of pulverized Polygonutum Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Quercus Bark

Quercus Cortex

ボクソク

Quercus Bark is the bark of *Quercus acutissima* Carruthers, *Quercus serrata* Murray, *Quercus mongolica* Fischer ex Ledebour var. *crispula* Ohashi or *Quercus variabilis* Blume (*Fagaceae*).

Description Plate-like or semi-tubular pieces of bark, 5 - 15 mm in thickness; externally grayish brown to dark brown, with thick periderm and longitudinal coarse splits; internally brown to light brown, with longitudinal ridges, the transverse section brown to light brown, white small spots composed of stone cells in groups observed sporadically.

Almost odorless, tasteless.

Under a microscope <*5.01*>, a transverse section reveals a cork layer with scattered cork stone cells; in secondary cortex fiber bundles lined almost stepwide, large groups of stone cells arranged irregularly; in parenchyma aggregate crystals of calcium oxalate scattered; adjacent to stone cells and fiber cells, cells containing solitary crystals of calcium oxalate observed, and these cells form crystal cell rows in a longitudinal section.

Identification To 2 g of pulverized Quercus Bark, add 10 mL of ethyl acetate, shake for 10 minutes, and centrifuge to remove ethyl acetate. Add 10 mL of acetone to the residue, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <*2.03*>. 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 (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): Two consecutive fluorescent spots in different colors are observed at around R_f 0.4. Then, spray evenly diluted sulfuric acid on the plate, heat at 105ºC. Examine under ultraviolet light (main wavelength: 365 nm): one of these spots produces fluorescence.

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*> Not more than 8.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Add the following:

Royal Jelly

Apilac

ローヤルゼリー

Royal Jelly is the viscous liquid or its dried substance secreted by the secreting gland on the head of *Apis mellifera* Linné or *Apis cerana* Fabricius (*Apidae*).

It contains not less than 4.0% and not more than 8.0% of 10-hydroxy-2-(*E*)-decenoic acid, calculated on the basis of dried material.

Description Slightly viscous liquid or powder, milky white to light yellow in color. Odor, characteristic; taste, astringent and acid.

Identification To a portion of Royal Jelly, equivalent to 0.2 g of dried substance, add 5 mL of water, 1 mL of dilute hydrochloric acid and 10 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer, evaporate under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 2 mg of 10-hydroxy-2-(*E*)-decenoic acid 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 Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28) (7:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution has the same color tone and R_f value with the dark purple spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*> Proceed with a portion of Royal Jelly, equivalent to 1.0 g of the dried substance, according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with an amount of Royal Jelly, equivalent to 0.40 g of the dried substance according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> The slightly viscous liquid: Not less than 57.0% and not more than 77.0% (6 hours).

The powder: Not less than 7.0% and not more than 13.0% (6 hours)

Total ash <*5.01*> Not more than 4.0%, calculated on the dried basis.

Acid-insoluble ash <*5.01*> Not more than 0.5%, calculated on the dried basis.

Component determination Weigh a portion of Royal Jelly, equivalent to 0.2 g of the dried substance, add 20 mL of methanol, treat with ultrasonic waves for 30 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, take exactly 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 10-hydroxy-2-(*E*)-decenoic acid for component determination, dissolve in methanol to make exactly 100 mL. To exactly 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol 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 standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 10-hydroxy-2-(*E*)-decenoic acid to that of the internal standard.

$$\text{Amount (mg) of 10-hydroxy-2-(}E\text{)-decenoic acid} = W_S \times (Q_T/Q_S) \times (3/4)$$

W_S: Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid for component determination

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 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 water, methanol and phosphoric acid (550:450:1)

Flow rate: Adjust the flow rate so that the retention time of 10-hydroxy-2-(*E*)-decenoic acid 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, 10-hydroxy-2-(*E*)-decenoic acid 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 ratio of the peak area of 10-hydroxy-2-(E)-decenoic acid to that of the internal standard is not more than 1.0%.

Container and storage Containers—Tight containers.

Storage—At not exceeding 10°C

Saposhnikovia Root

ボウフウ

Add the following next to the Description:

Identification To 1 g of pulverized Saposhnikovia Root, add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glucosyl-5-*O*-methylvisamminol 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 Thin-layer Chromatography *<2.03>*. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (10:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among several spots from the sample solution has the same color tone and R_f value with the blue spot from the standard solution.

Scopolia Rhizome

ロートコン

Change the Purity to read:

Purity (1) Heavy metals *<1.07>* Proceed with 3.0 g of pulverized Scopolia Rhizome according to Method 3, and perform the test. Prepare the control solution with 4.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic *<1.11>* Prepare the test solution with 0.40 g of pulverized Scopolia Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Senega

セネガ

Change the Purity to read:

Purity (1) Stem—The amount of the stems contained in Senega does not exceed 2.0%.

(2) Heavy metals *<1.07>* Proceed with 3.0 g of pulverized Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic *<1.11>* Prepare the test solution with 0.40 g of pulverized Senega according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter *<5.01>* The amount of foreign matter other than the stems is not more than 1.0%.

Powdered Senega

セネガ末

Change the Purity to read:

Purity (1) Heavy metals *<1.07>* Proceed with 3.0 g of Powdered Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic *<1.11>* Prepare the test solution with 0.40 g of Powdered Senega according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope *<5.01>*, stone cells, starch grains or crystals of calcium oxalate are not observed.

Add the following:

Shimbuto Extract

真武湯エキス

Shimbuto Extract contains not less than 26 mg and not more than 78 mg of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 0.8 g of Ginger) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride) or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and 14-benzoylhypacomine hydrochloride) or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) per the extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Rhizome, 1 g of Ginger and 1 g Processed Aconit Root 1 of "Processed Aconit Root", or with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Lancea Rhizome, 1 g of Ginger and 1 g of Powdered Processed Aconit Root 1 of "Powdered Processed Aconit Root", or with 5 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Rhizome, 0.8 g of Ginger and 1 g of Powdered Processed Aconit Root 2 of "Powdered Processed Aconit Root",

or with 4 g of Poria Sclerotium, 3 g of Paeony Root, 3 g of Atractylodes Lancea Rhizome, 1.5 g of Ginger and 0.5 g of Powdered Processed Aconit Root 1 of "Powdered Processed Aconit Root".

Description Shimbuto Extract occurs as light yellow-brown to brown powder. It has slightly a characteristic odor and a hot and bitter taste.

Identification **(1)** To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution (Peony Root).

(2) (For preparation prescribed Atractylodes Rhizome) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and R_f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) (For preparation prescribed Atractylodes Lancea Rhizome) To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 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 hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around R_f 0.4. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes and allowed to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the blue-green spot from the standard solution (Ginger).

(5) To 3.0 g of Shimbuto Extract, add 20 mL of diethyl ether and 2 ml of ammonia TS, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the supernatant liquid under reduced pressure, add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of 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 Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

Purity **(1)** Heavy metals <*1.07*> Prepare the test solution with 1.0g of Shimbuto Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*> Prepare the test solution with 0.67 g of Shimbuto Extract according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a

mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

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 phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying *<2.41>* Not more than 7.0% (1 g, 105°C, 5 hours).

Total ash *<5.01>* Not more than 10.0%.

Assay (1) Peoniflorin—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water), and 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of peoniflorin.

$$\text{Amount (mg) of peoniflorin } (C_{23}H_{28}O_{11}) = W_S \times (A_T / A_S) \times (1/2)$$

W_S: Amount (mg) of Peoniflorin 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: 1.0 mL per minute (the retention time of peoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this standard solution under the above operating conditions, albiflorin and peoniflorin 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 peoniflorin is not more than 1.5%.

(2) [6]-gingerol—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for component determination, dissolve in diluted methanol to make exactly 100 mL. Pipet 5 mL of this solution, 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 standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol.

$$\text{Amount (mg) of [6]-gingerol} = W_S \times (A_T / A_S) \times (1/20)$$

W_S: Amount (mg) of [6]-gingerol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 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 phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol 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 factor of the peak of [6]-gingerol 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 [6]-gingerol is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for component determination as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

Amount (mg) of benzoylmesaconine hydrochloride
$= C_{SM} \times (A_{TM} / A_{SM}) \times 10$

Amount (mg) of benzoylhypaconine hydrochloride
$= C_{SH} \times (A_{TH} / A_{SH}) \times 10$

Amount (mg) of 14-anisoylaconine hydrochloride
$= C_{SA} \times (A_{TA} / A_{SA}) \times 10$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution TS for component determination

C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for component determination in aconitum monoester alkaloids standard solution for TS component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylhypaconine and benzoylmesaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for component determination under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Container and storage Containers—Tight containers.

Turmeric

ウコン

Change the Origin and the identification to read:

Turmeric is the rhizome of *Curcuma Longa* Linné (*Zingiberaceae*) with or without cork layers, usually with the application of blanching.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

Identification **(1)** To 0.5 g of pulverized Turmeric, add 20 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 Thin-layer Chromatography <*2.03*>. 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, hexane and acetic acid (100) (70:30:1) to a distance about 10 cm, and air-dry the plate: a yellow spot appears at around R_f 0.4.

(2) To 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), centrifuge after shaking for 20 minutes, and separate the supernatant liquid. Perform the test as directed in the Component Determination, and measure the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

Add the following next to Extract Content:

Component determination Weigh accurately about 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), and proceed in the same manner as described above. Combine all the extracts,

add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for component determination, and dissolve in methanol to make exactly 50 mL. Weigh accurately 10 mL of this solution, 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 standard solution as described under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_{TC}, A_{TD} and A_{TD} of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A_S of curcumin in the standard solution.

Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin)
$= W_S \times \{(A_{TC} + A_{TD} + A_{TB} \times 0.69) / A_S\} \times (1 / 5)$

W_S: Amount (mg) of curcumin 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 15 cm in length, packed with octadecylsilianized 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 acetic acid (100) (56:43:1).

Flow rate: 1.0 mL per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin, demethoxycurcumin and bisdemethoxycurcumin for component determination in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcumin are eluted in this order with the resolution among 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 curcumin is not more than 1.5%.

Powdered Turmeric

ウコン末

Change the Origin and the Identification to read:

Powdered Turmeric is the powder of Turmeric.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

Identification **(1)** To 0.5 g of Powdered Turmeric, add 20 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 Thin-layer Chromatography *<2.03>*. 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, hexane and acetic acid (100) (70:30:1) to a distance about 10 cm, and air-dry the plate: a yellow spot appears at around R_f 0.4.

(2) To 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), centrifuge after shaking for 20 minutes, and separate the supernatant liquid. Perform the test as directed in the Component Determination, and measure the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

Add the following next to Extract Content:

Component determination Weigh accurately about 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for component determination, and dissolve in methanol to make exactly 50 mL. Weigh accurately 10 mL of this solution, 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 standard solution as described under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_{TC}, A_{TD} and A_{TB} of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A_S of curcumin in the standard solution.

Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin
$= W_S \times \{(A_{TC} + A_{TD} + A_{TB} \times 0.69) / A_S\} \times (1 / 5)$

W_S: Amount (mg) of curcumin 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 15 cm in length, packed with octadecylsilianized 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 acetic acid (100) (56:43:1).

Flow rate: 1.0 mL/per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin, demethoxycurcumin and bisdemethoxycurcumin for component determination in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcu-

min are eluted in this order with the resolution among 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 curcumin is not more than 1.5%.

Zanthoxylum Fruit

サンショウ

Change the Total ash to read:

Total ash <*5.01*> Not more than 8.0%.

Powdered Zanthoxylum Fruit

サンショウ末

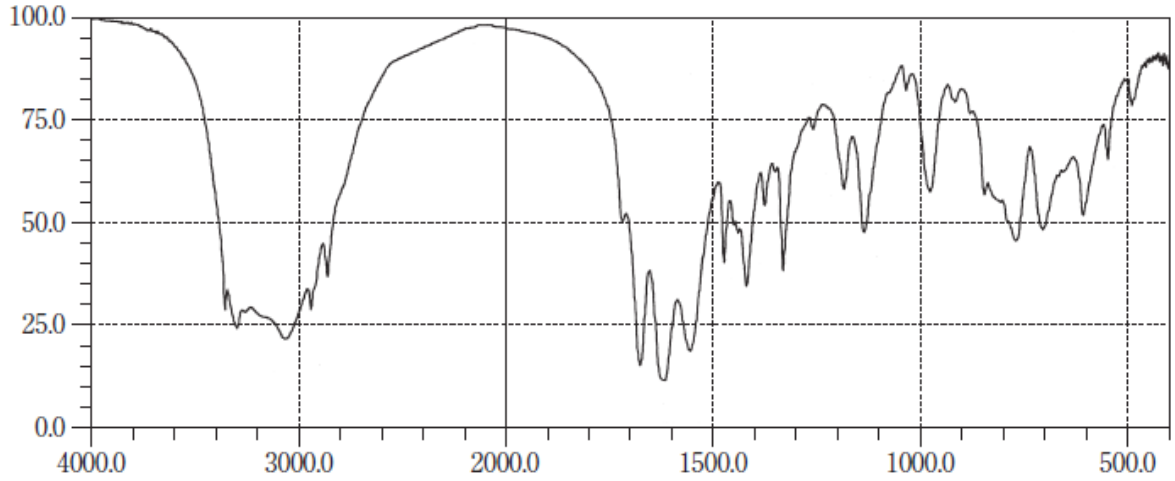
Change the Total ash to read:

Total ash <*5.01*> Not more than 8.0%

Infrared
Reference Spectra

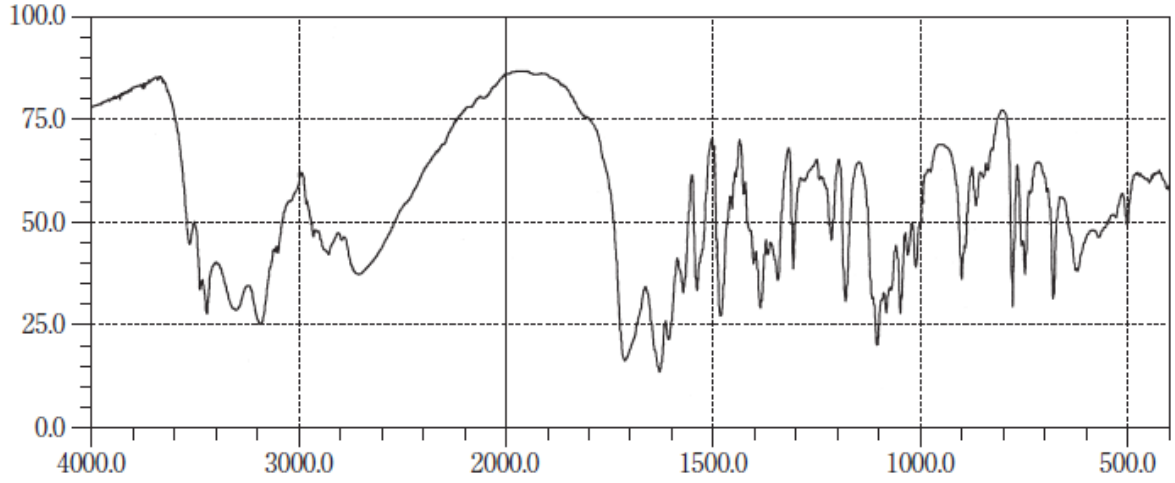
Change to read the following Infrared Reference Spectrum:

L-Arginine

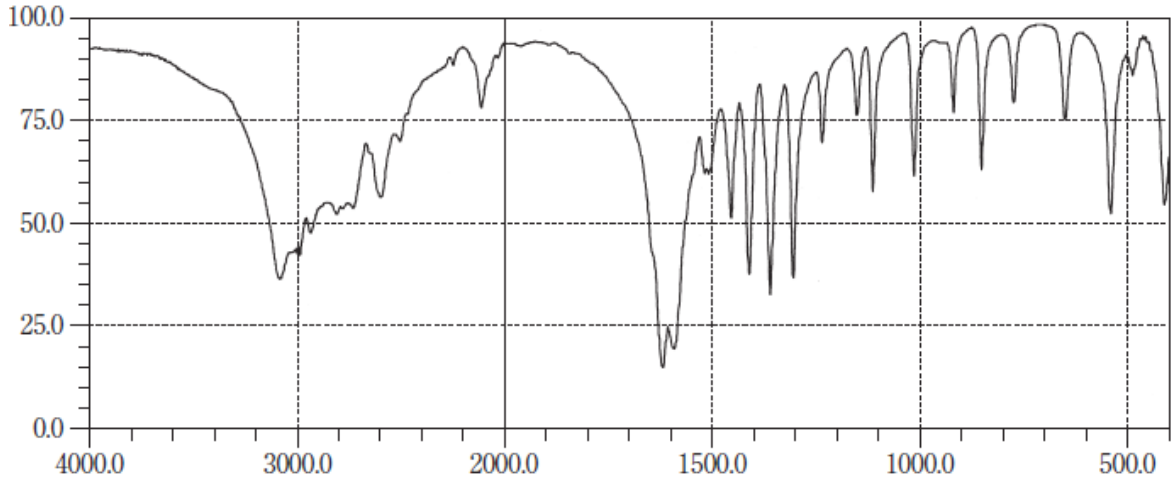


Add the following 51 spectra:

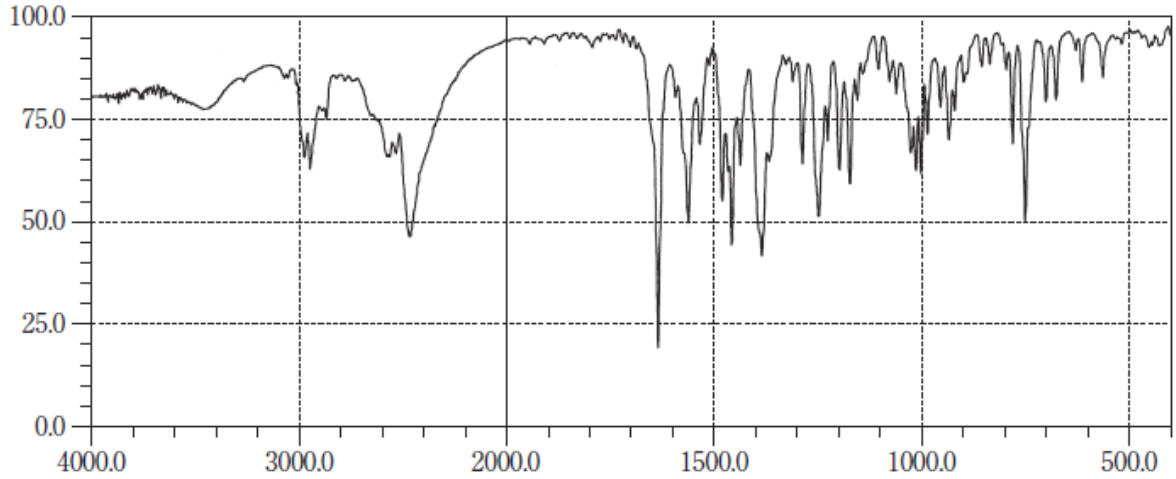
Aciclovir



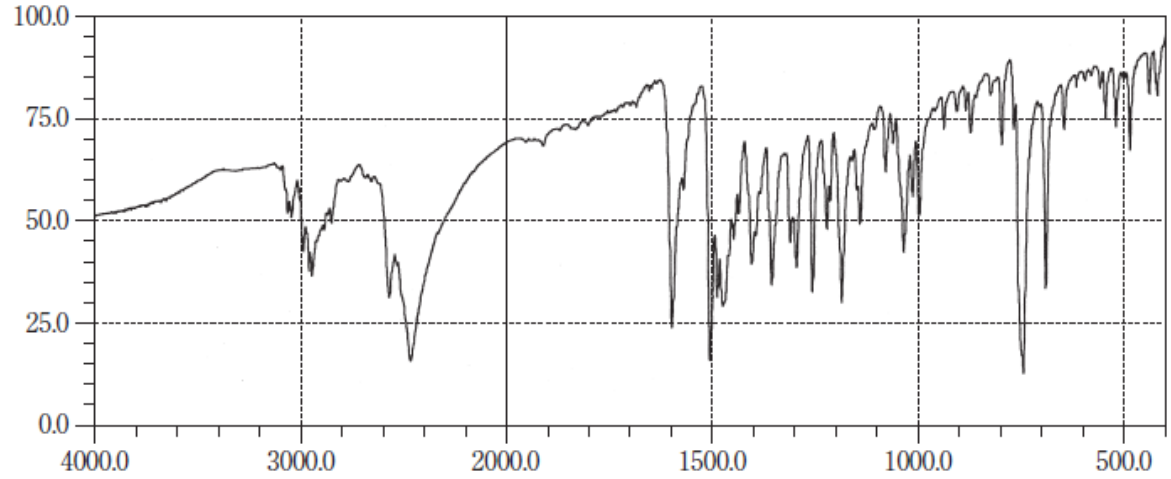
L-Alanine



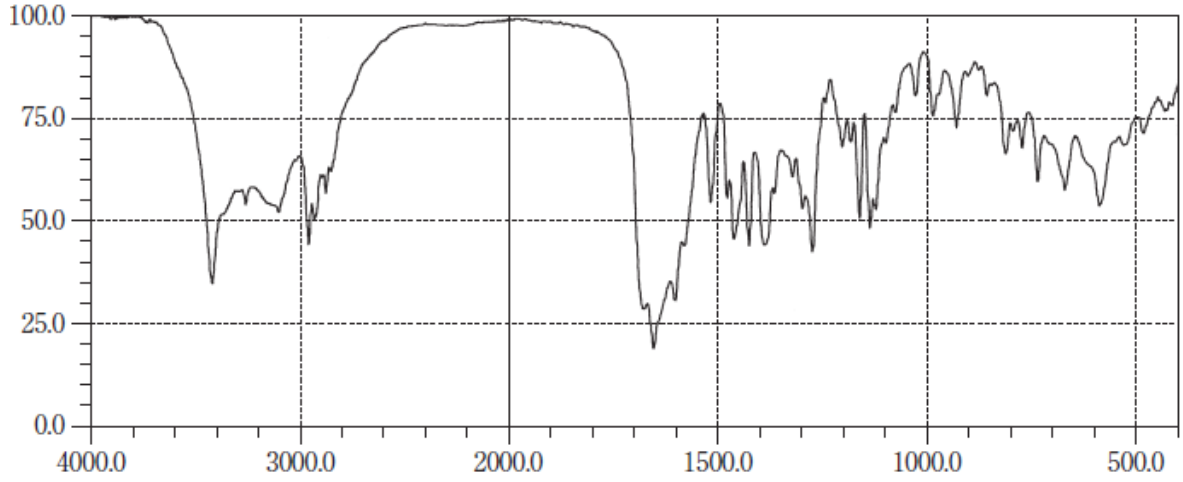
Amiodarone Hydrochloride



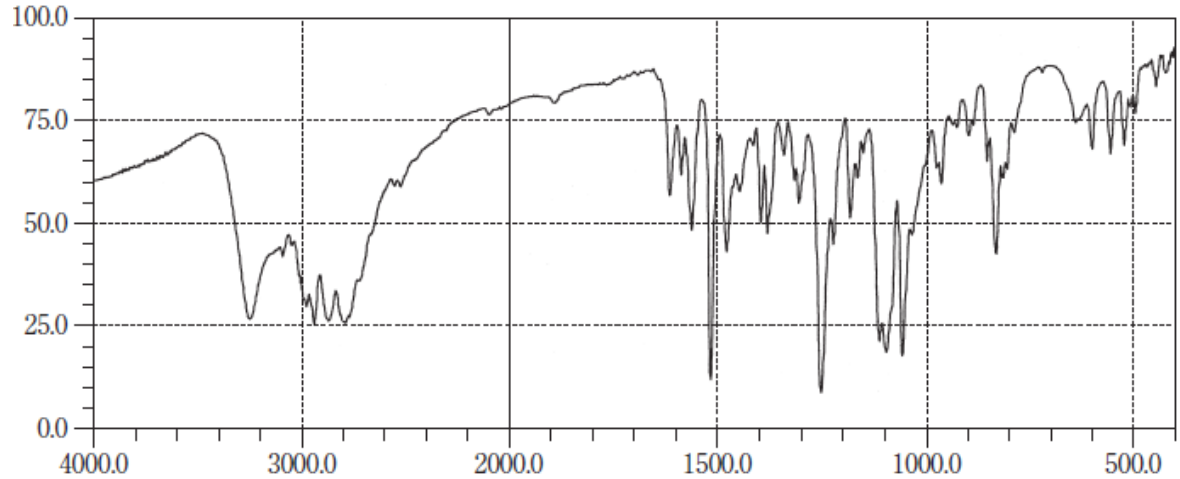
Aprindine Hydrochloride



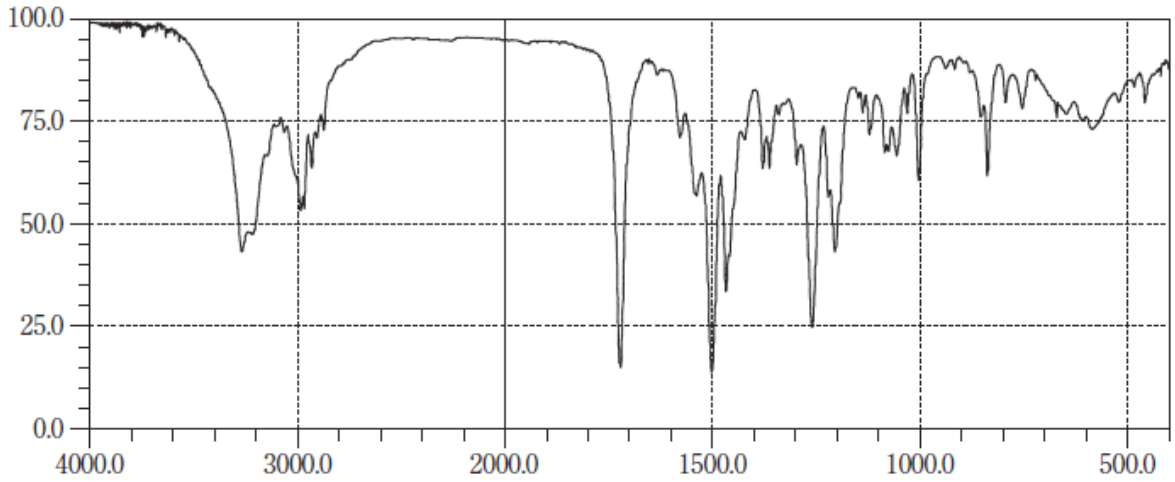
Argatroban Hydrate



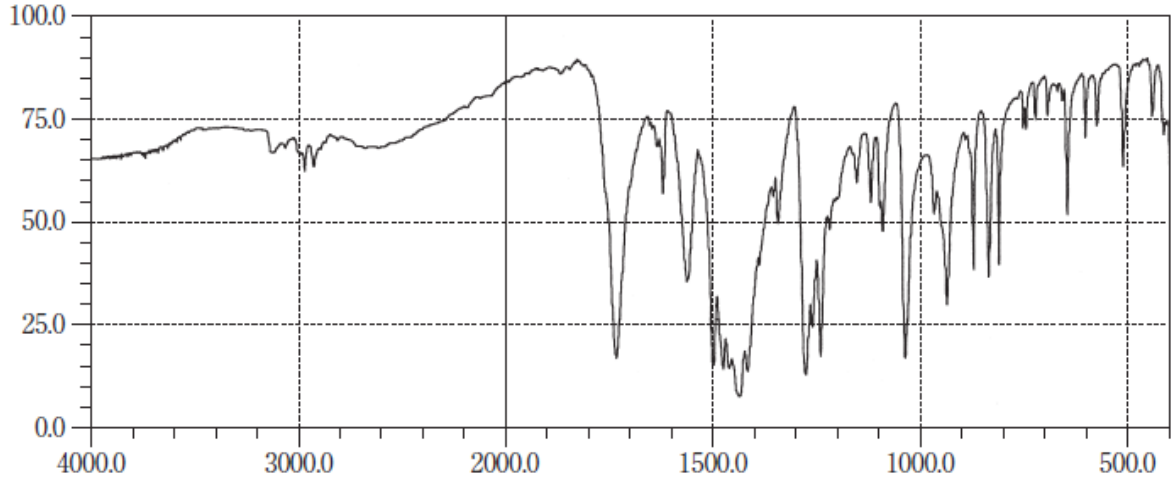
Betaxolol Hydrochloride



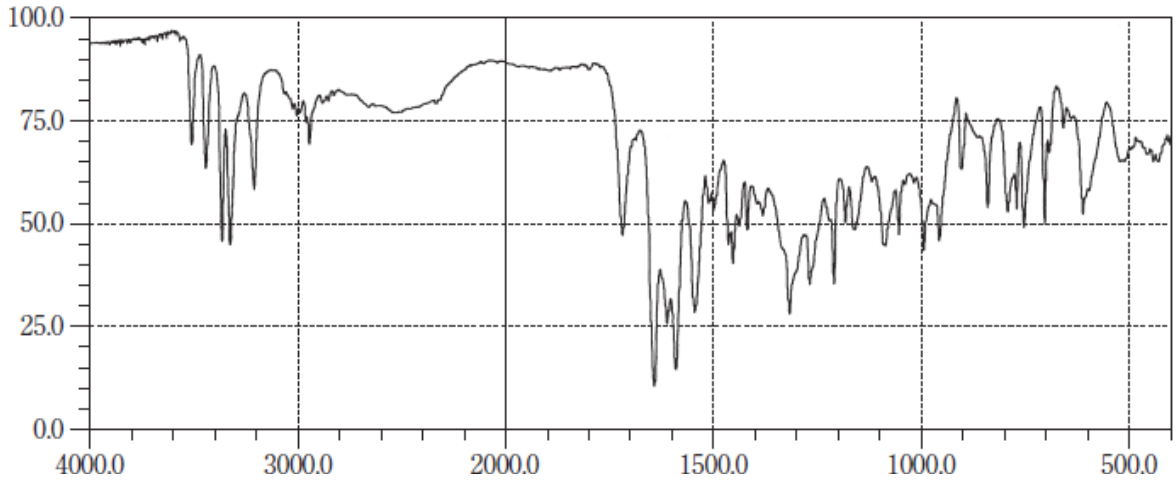
Cadralazine



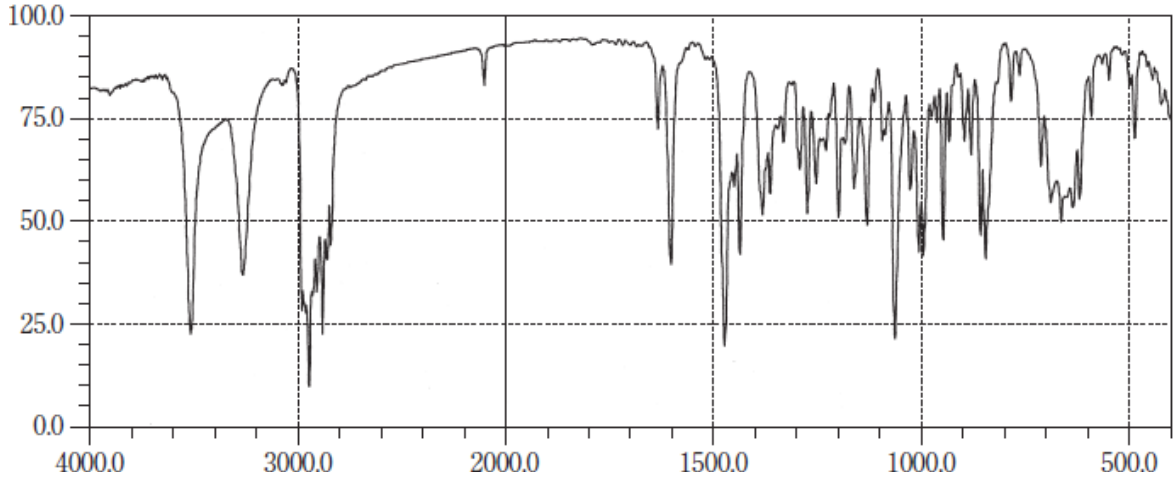
Cinoxacin



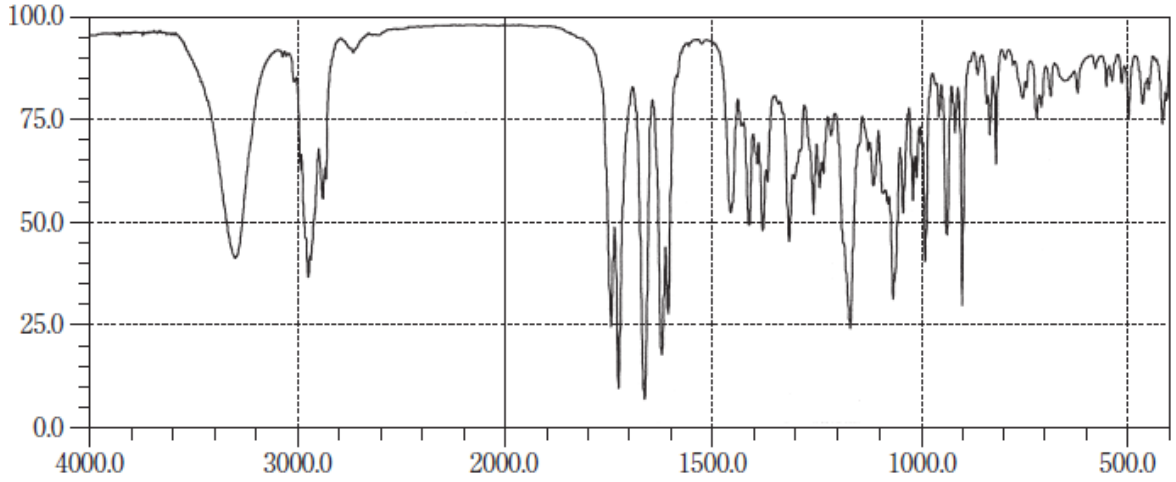
Clebopride Malate



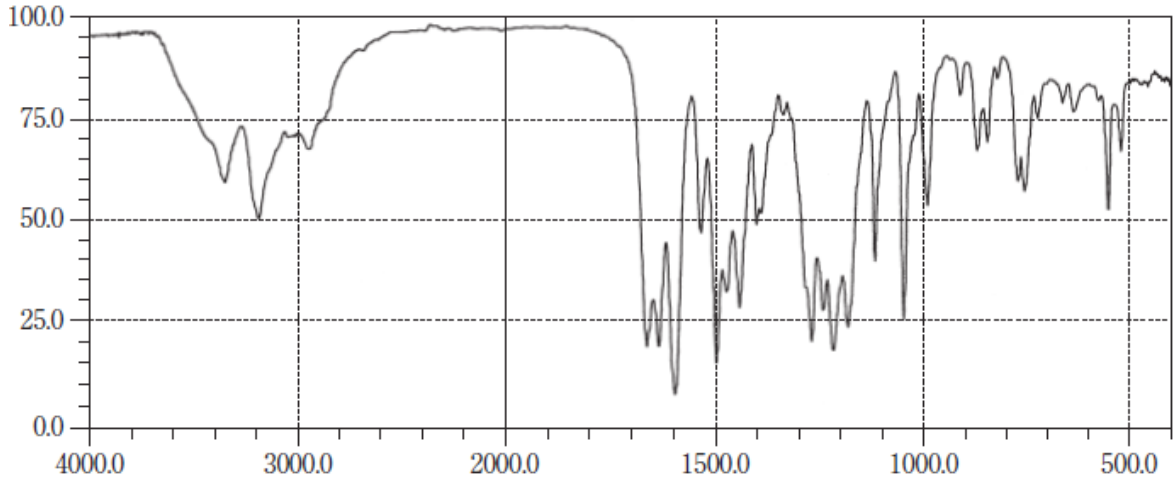
Danazol



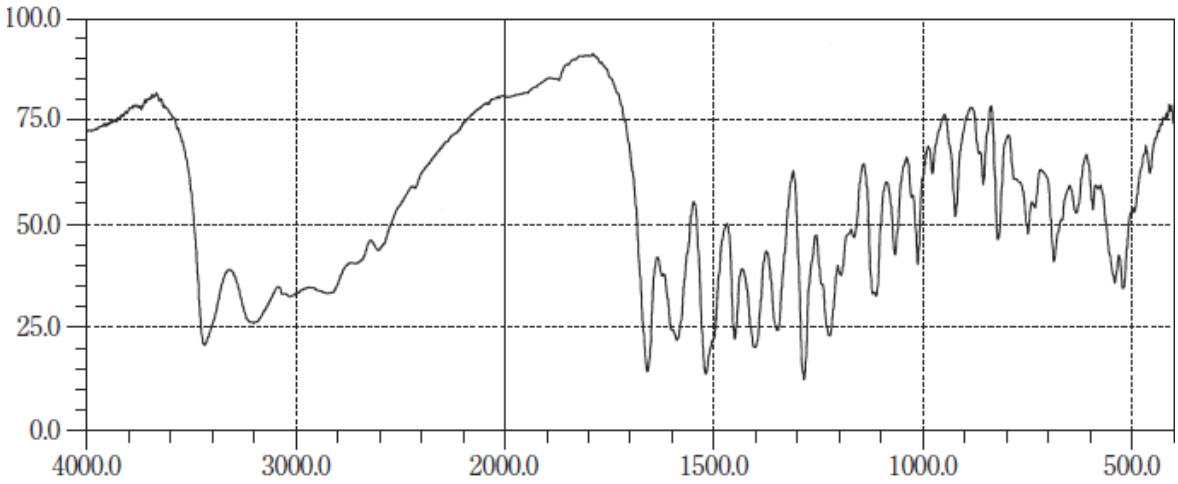
Diflucortolone Valerate



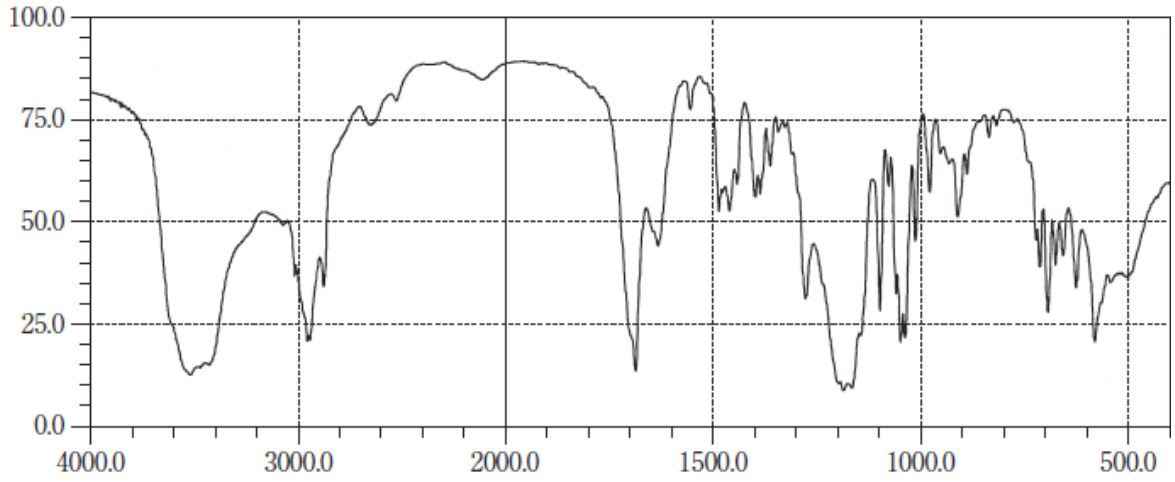
Doxazosin Mesilate



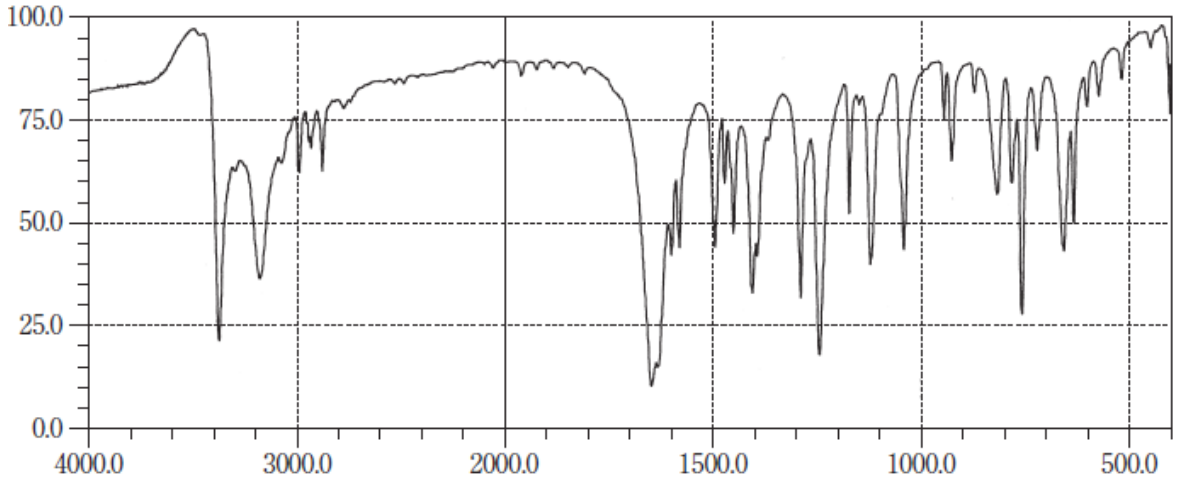
Droxidopa



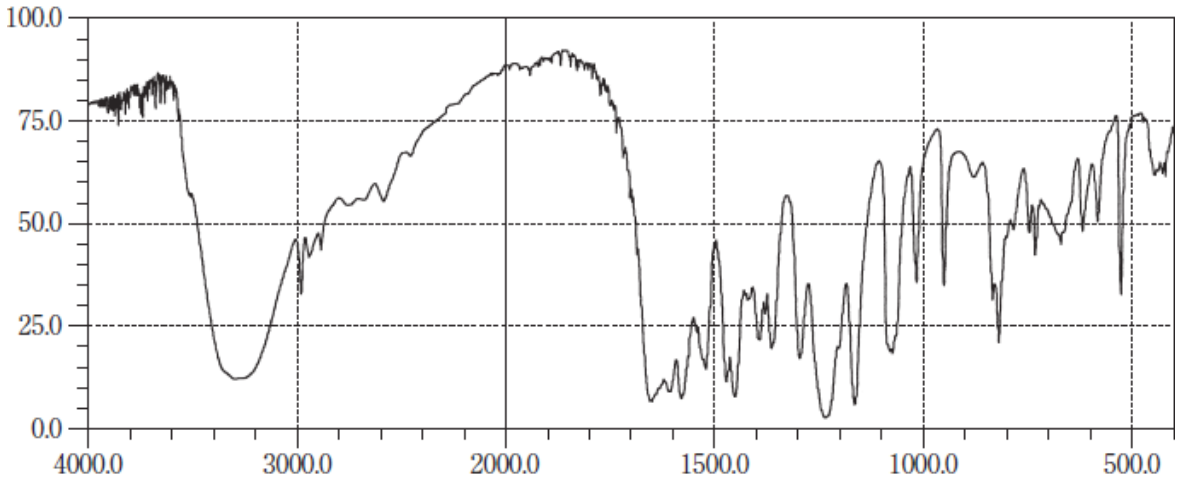
Ecabet Sodium Hydrate



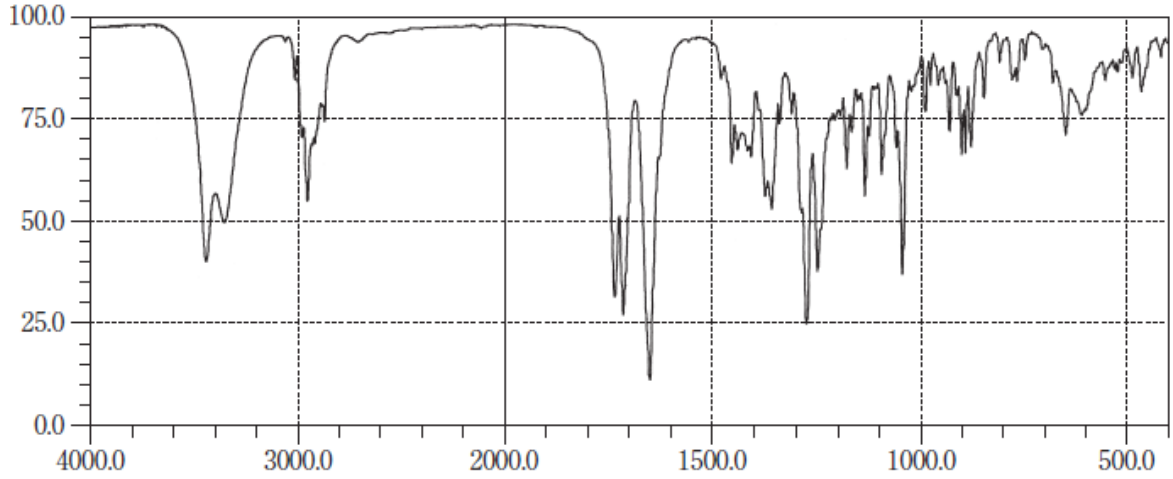
Ethenzamide



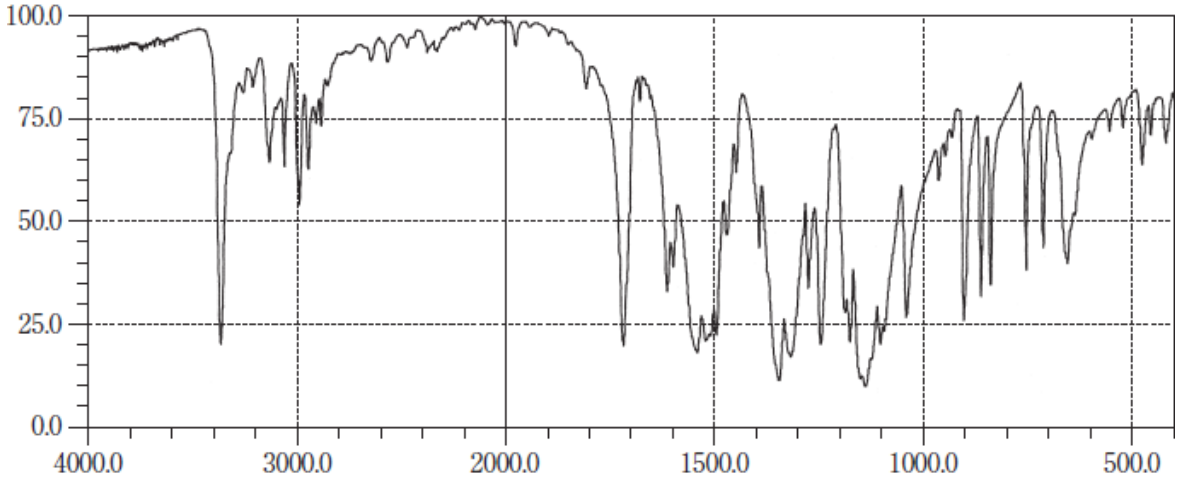
Flopropione



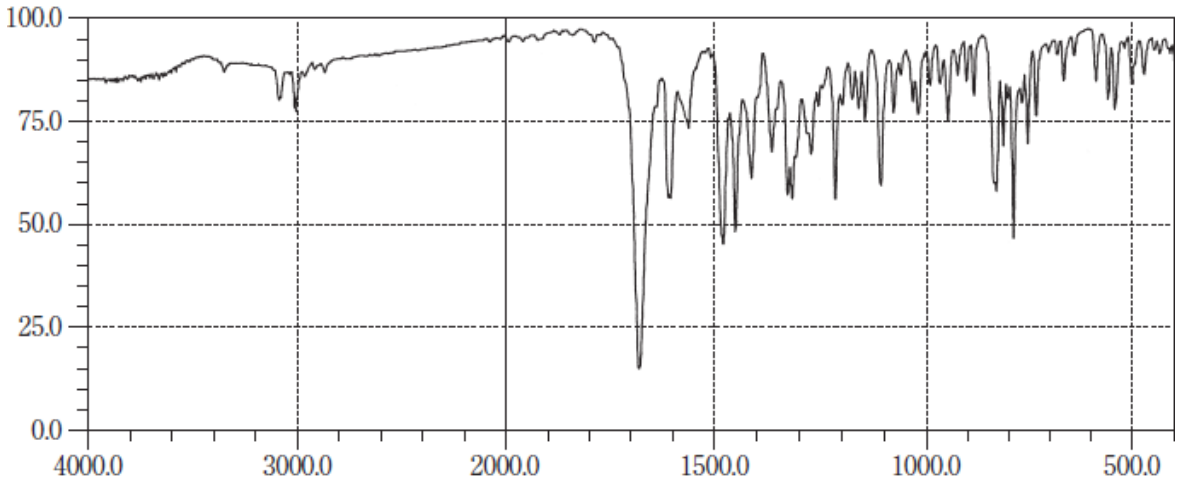
Fludrocortisone Acetate



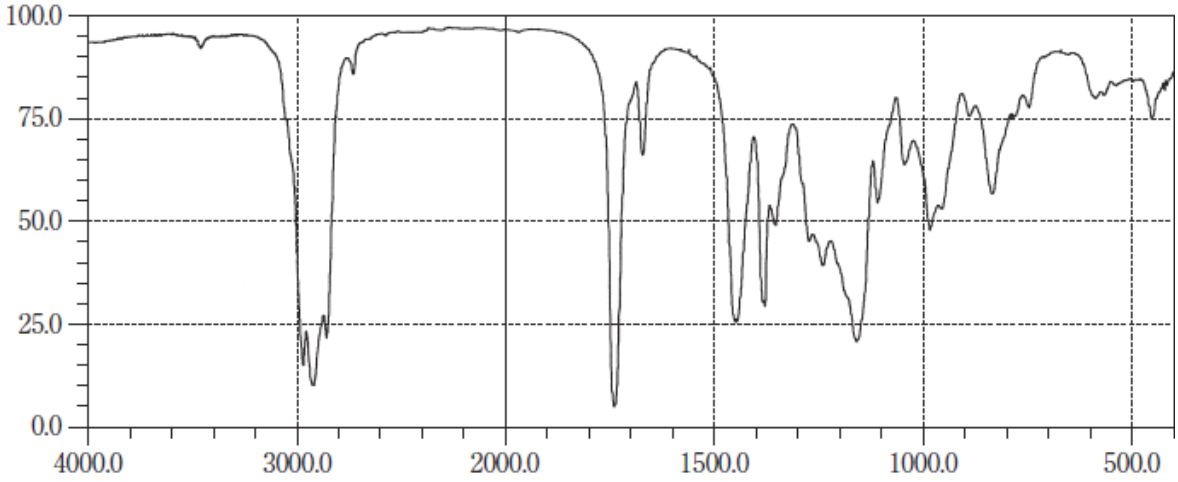
Flutamide



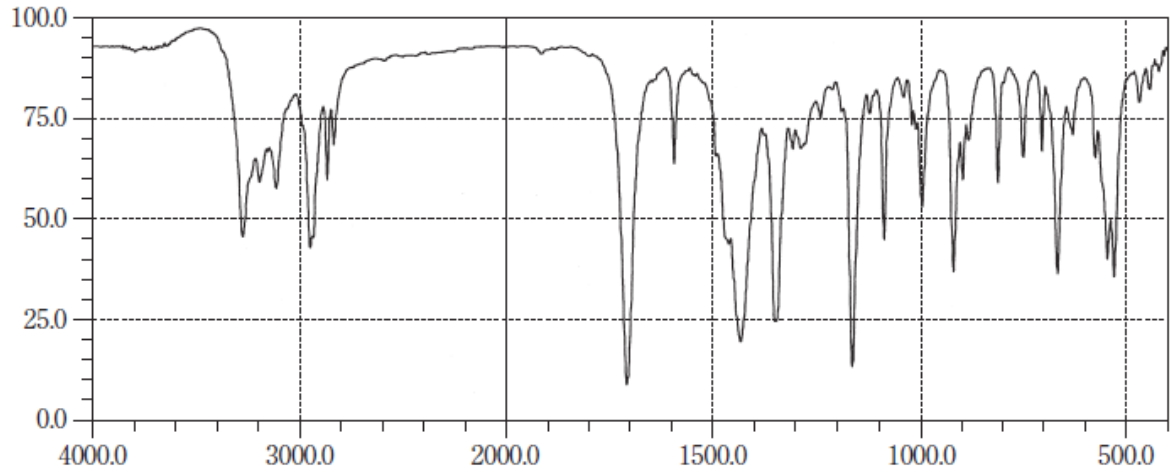
Flutoprazepam



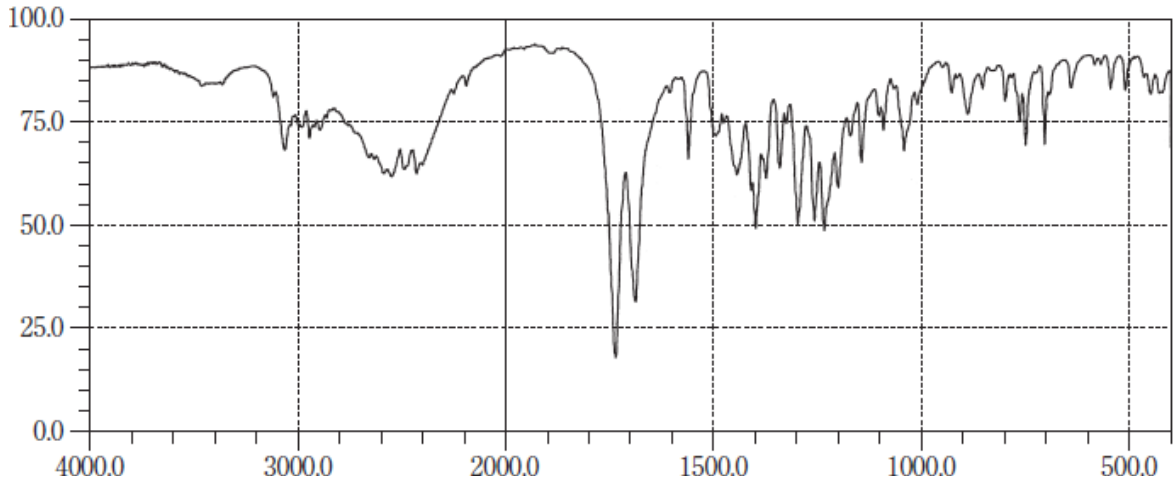
Gefarnate



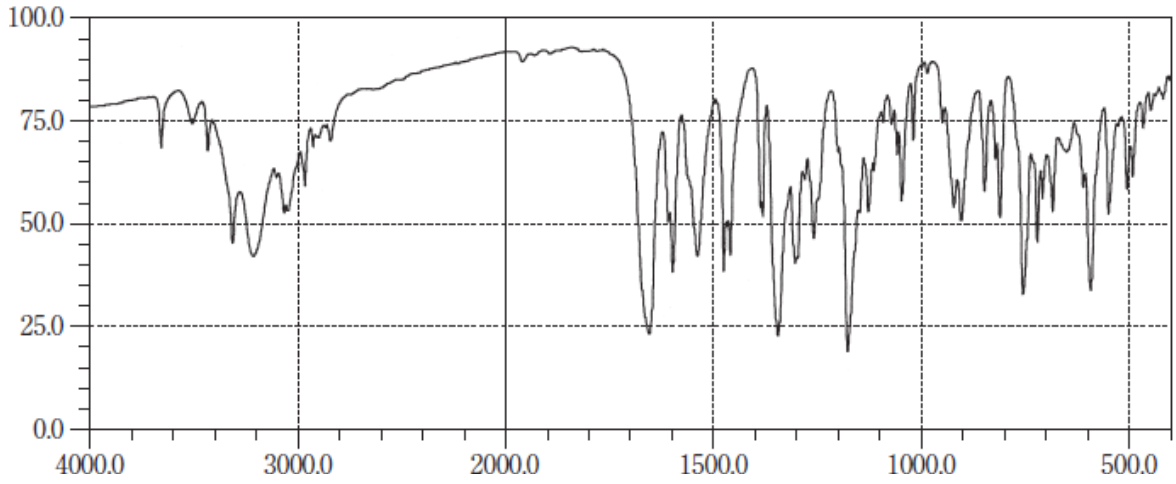
Gliclazide



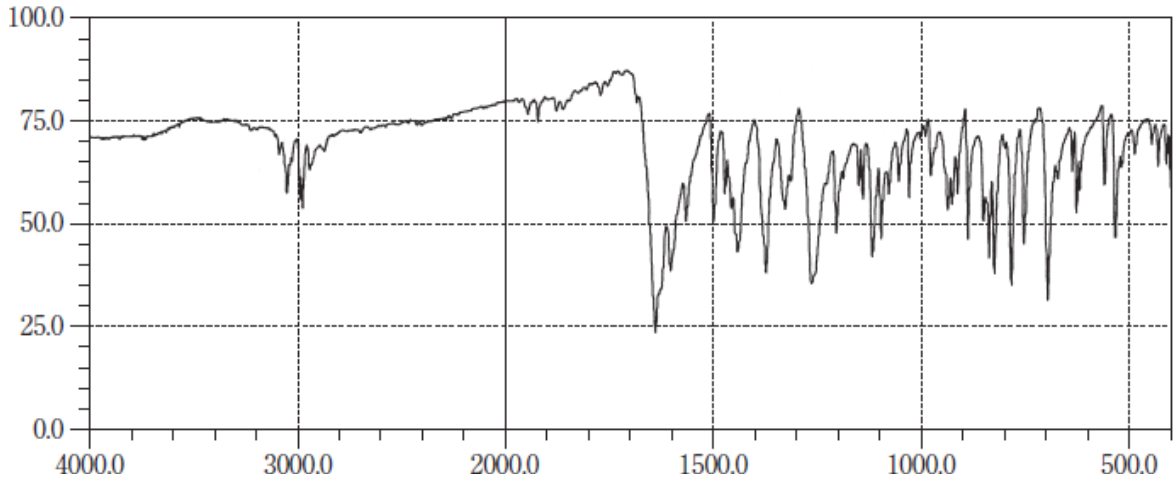
Imidapril Hydrochloride



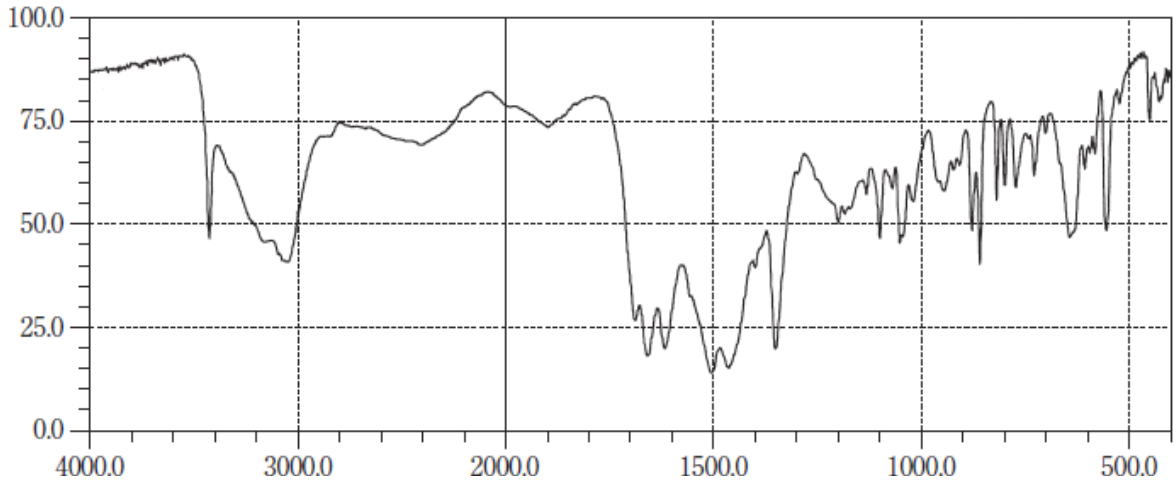
Indapamide



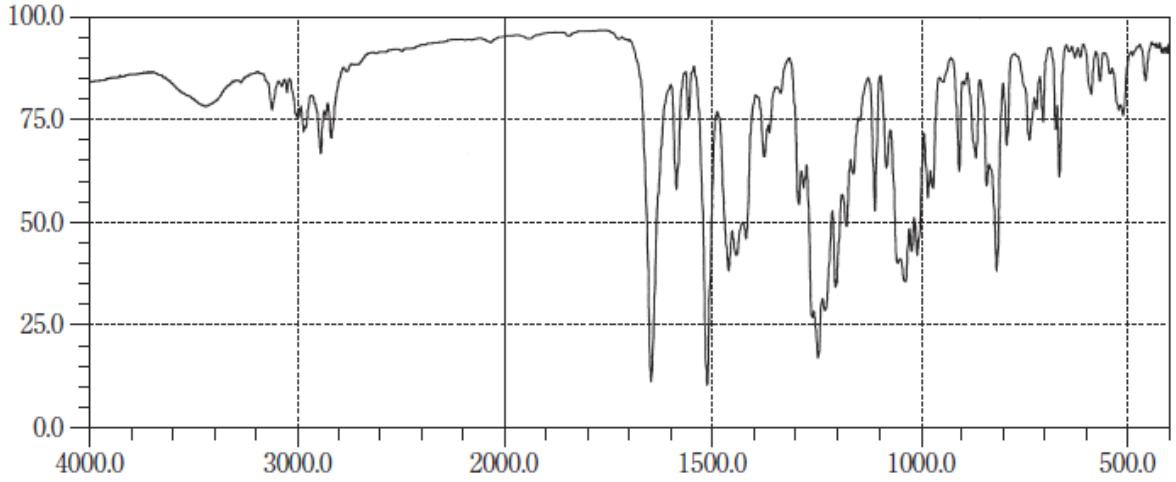
Ipriflavone



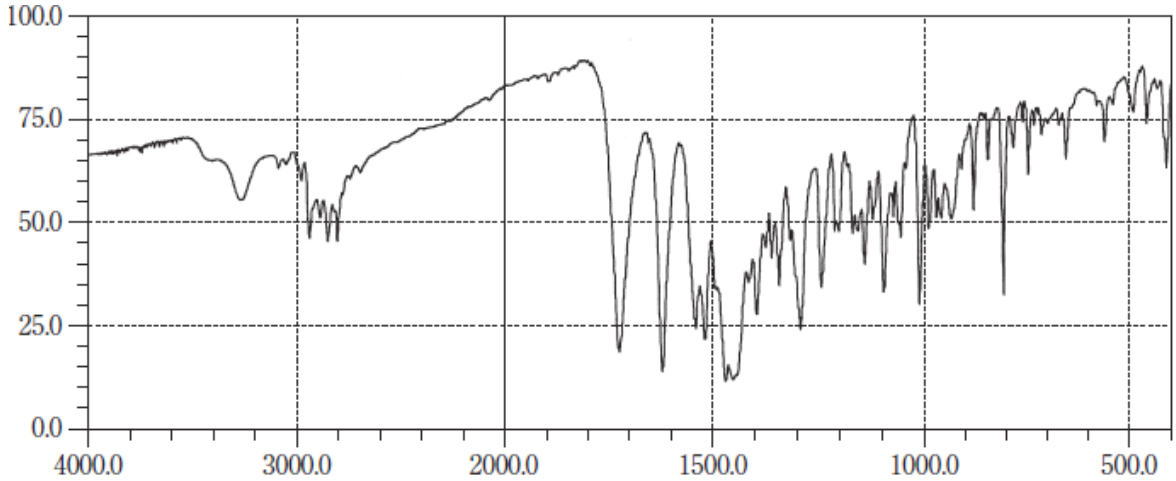
Irsogladine Maleate



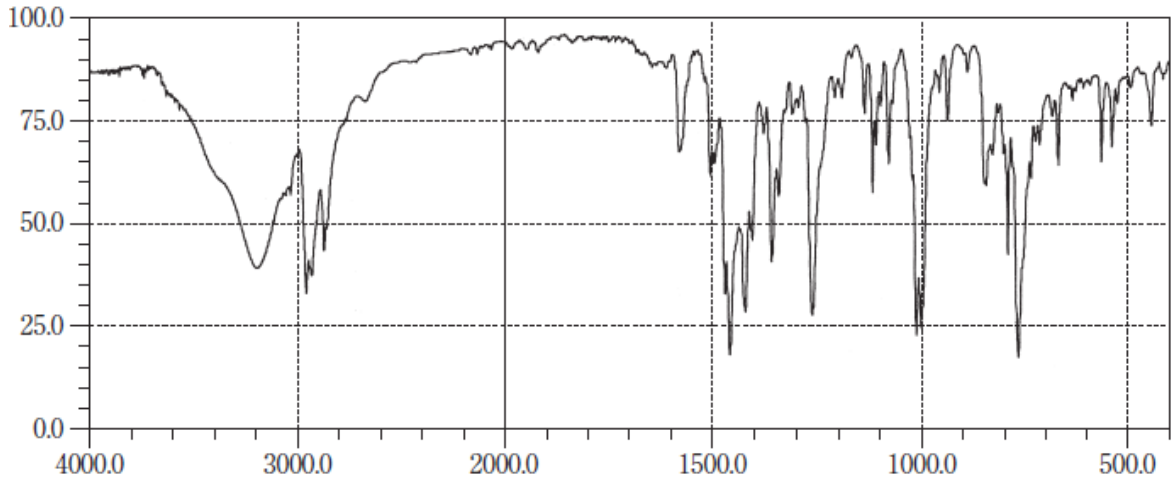
Ketoconazole



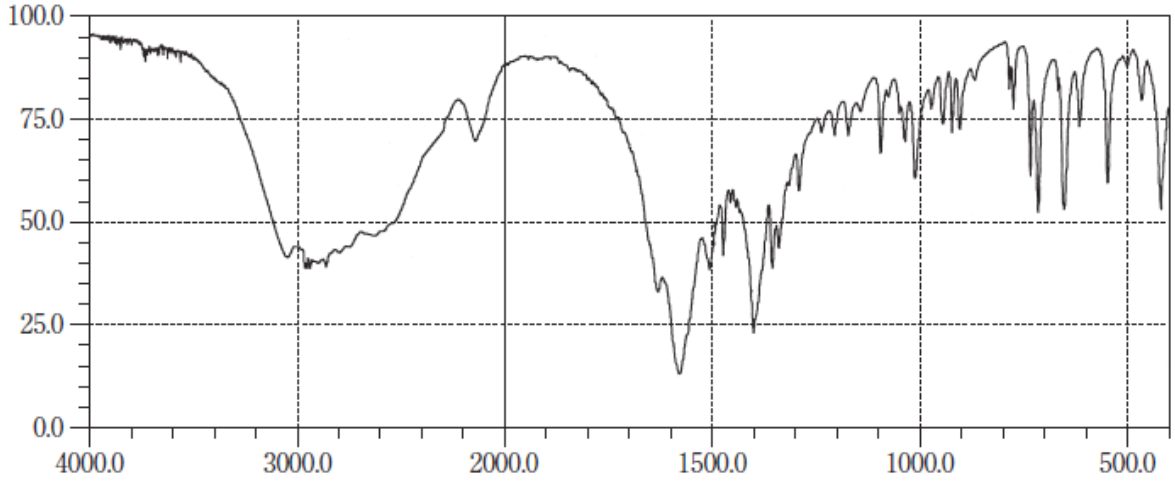
Levofloxacin Hydrate



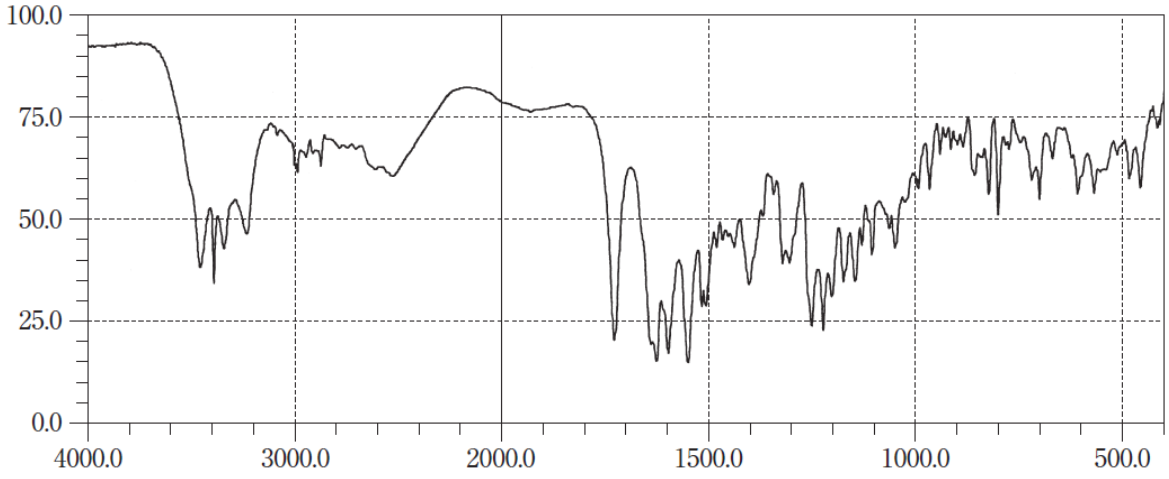
Losartan Potassium



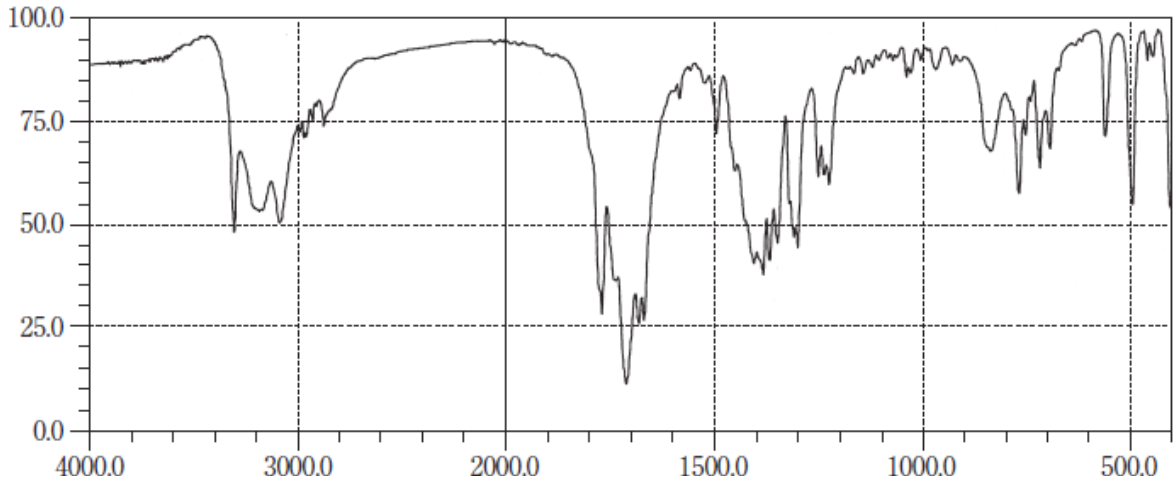
L-Lysine Acetate



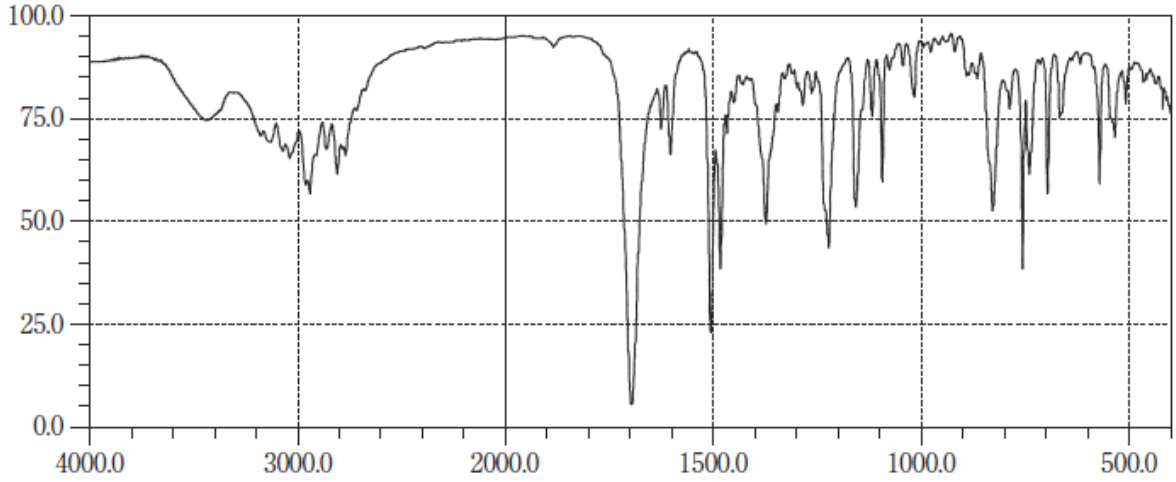
Mosapride Citrate Hydrate



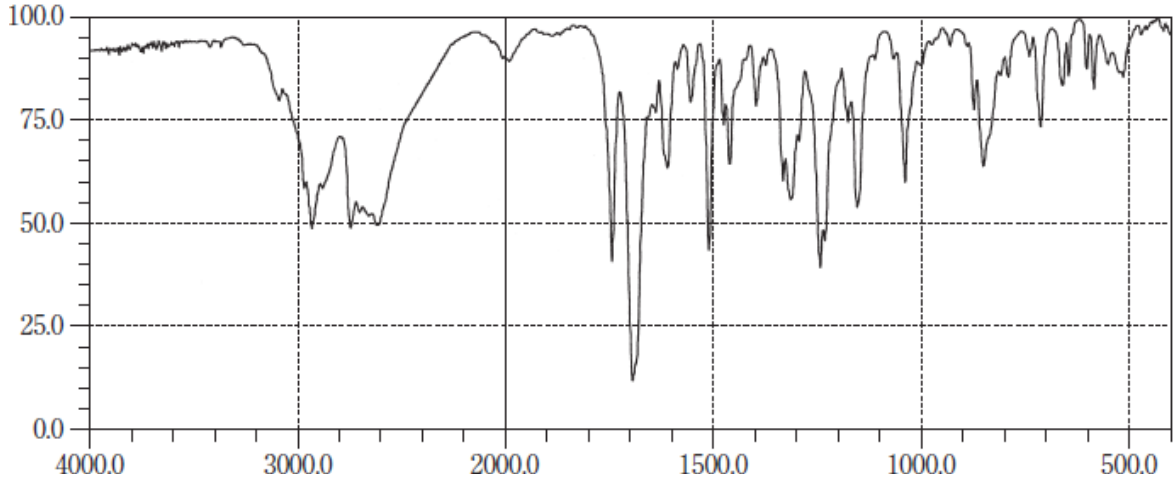
Phenobarbital



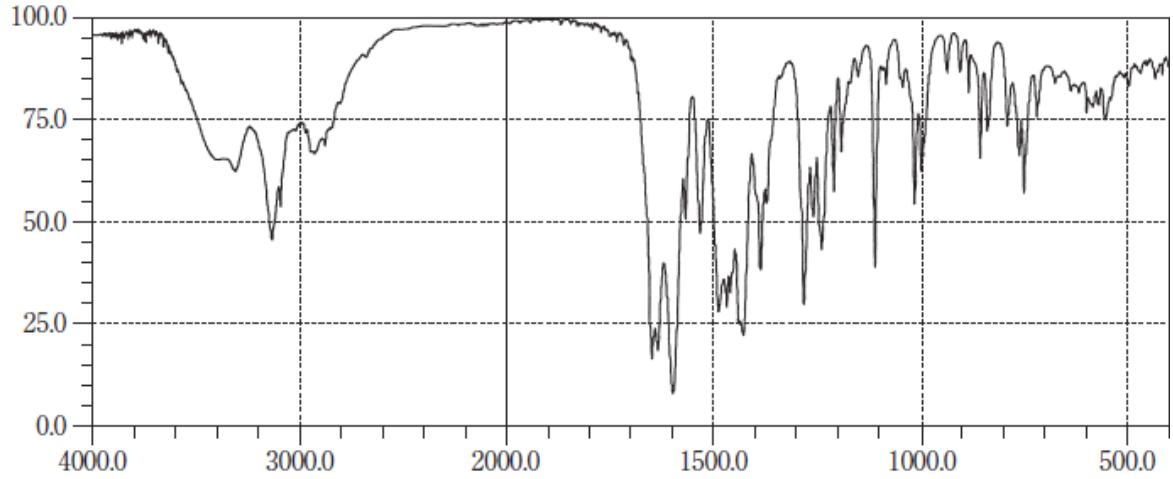
Pimozide



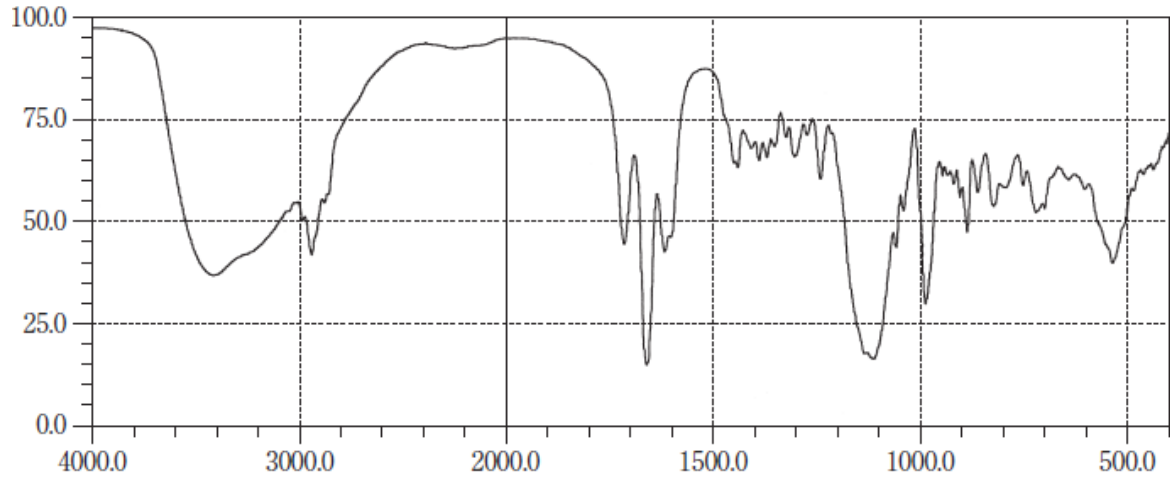
Pioglitazone Hydrochloride



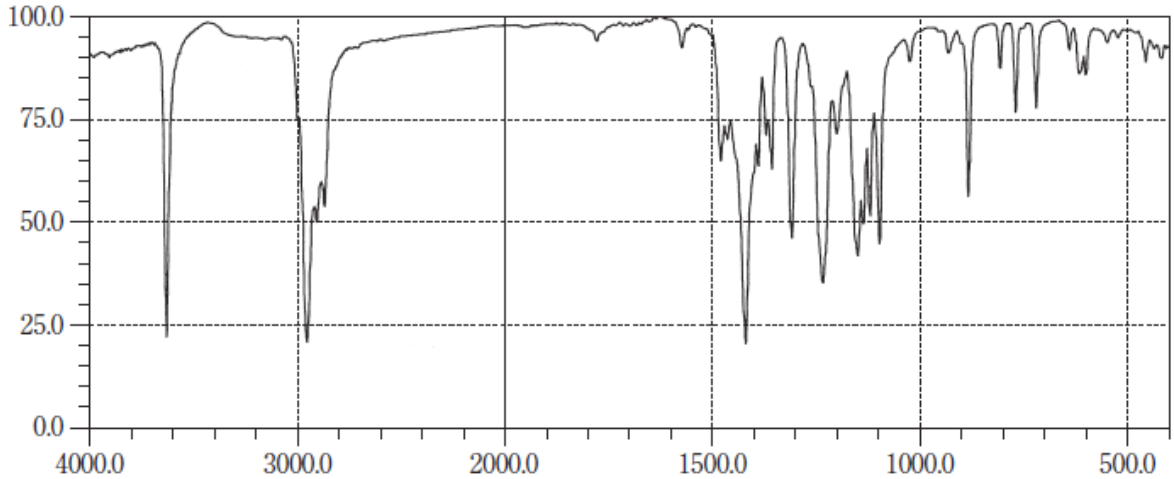
Prazosin Hydrochloride



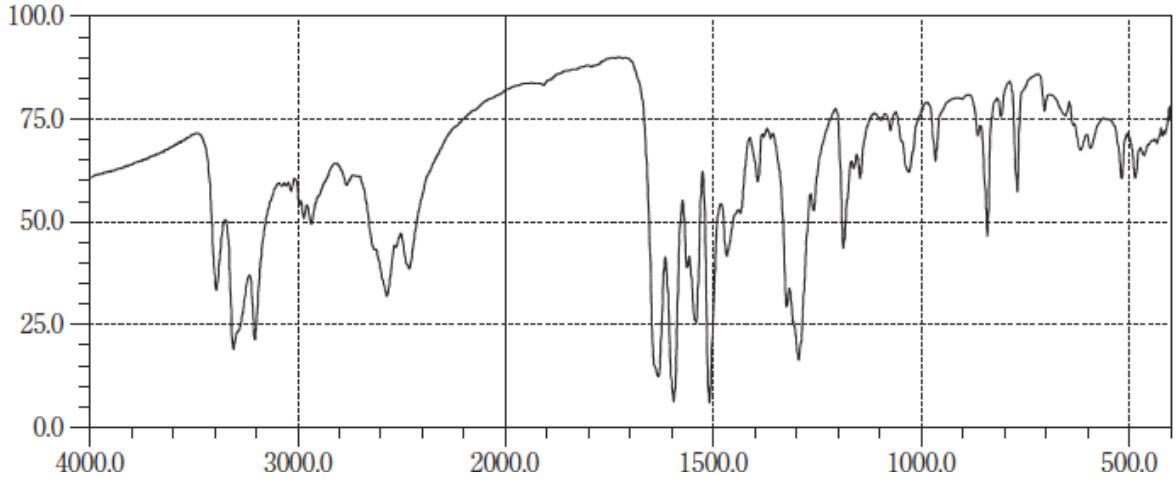
Prednisolone Sodium Phosphate



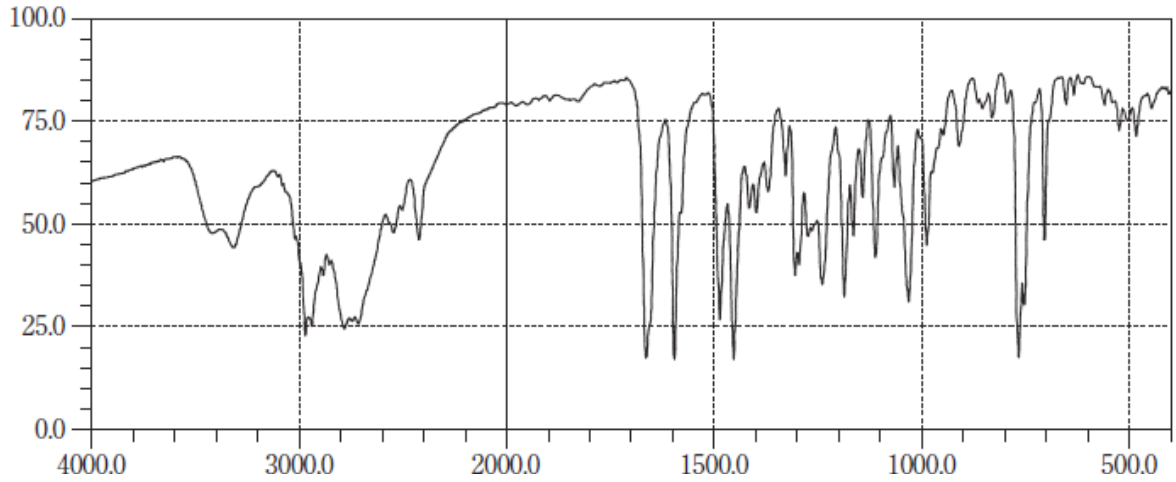
Probucol



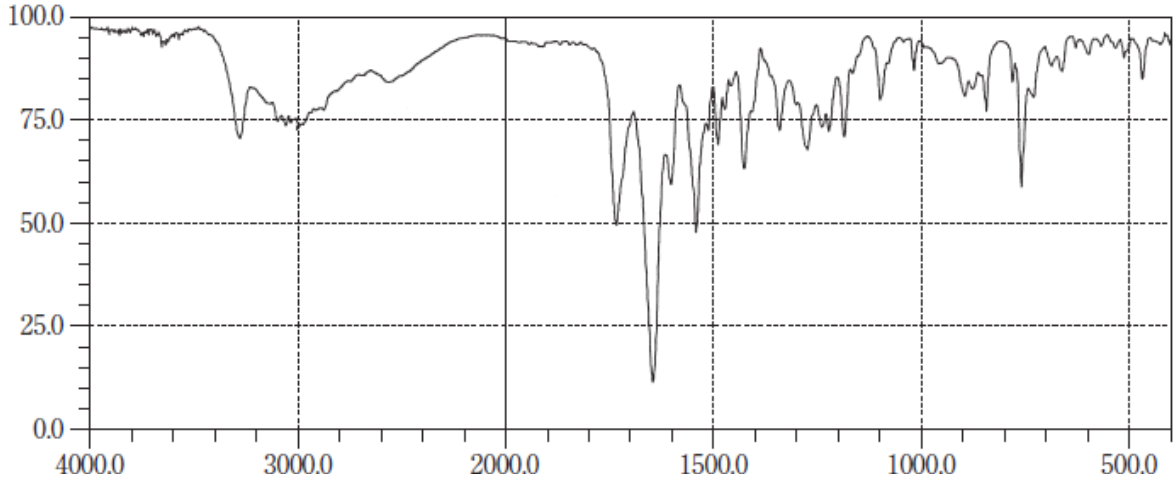
Procainamide Hydrochloride



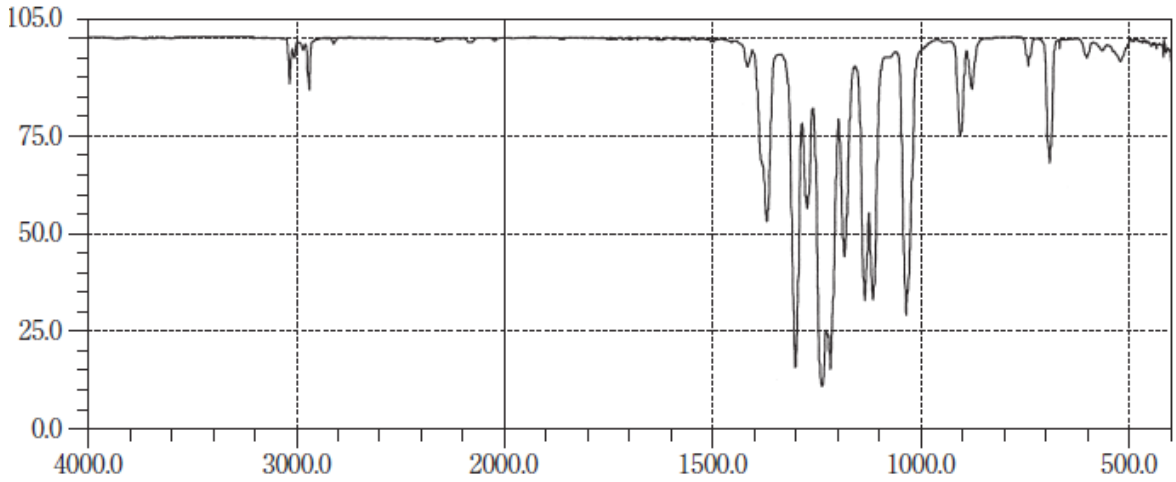
Propafenone Hydrochloride



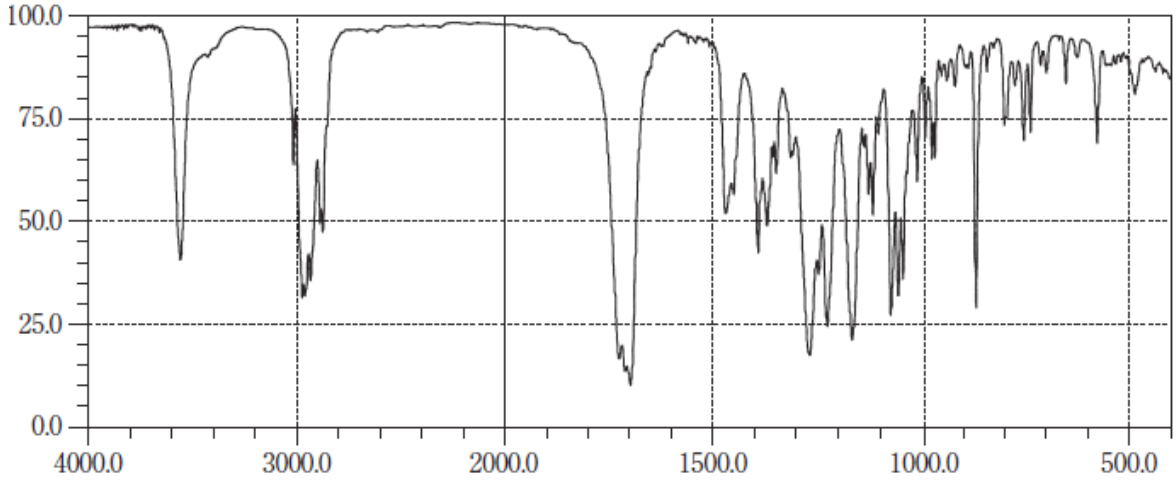
Rebamipide



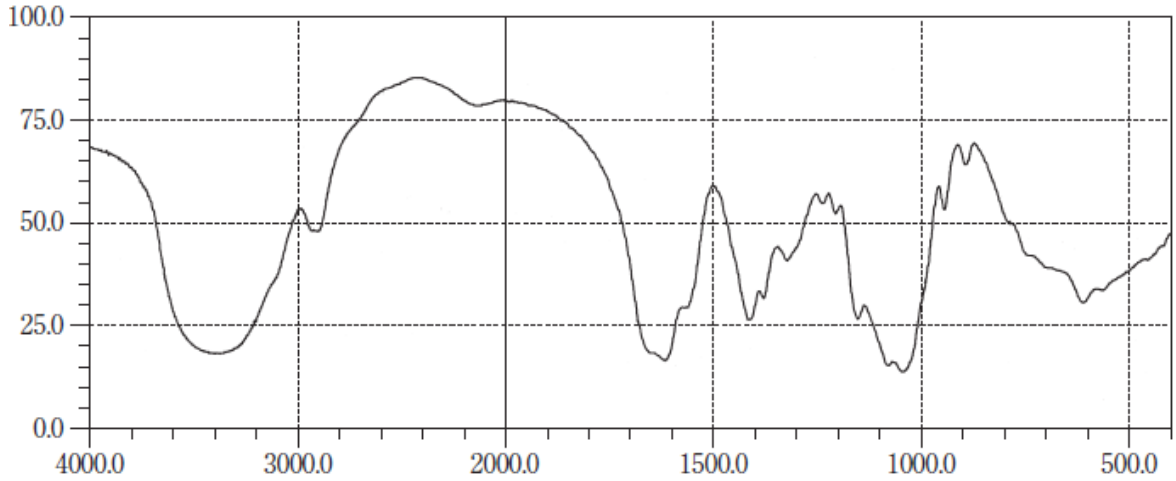
Sevoflurane



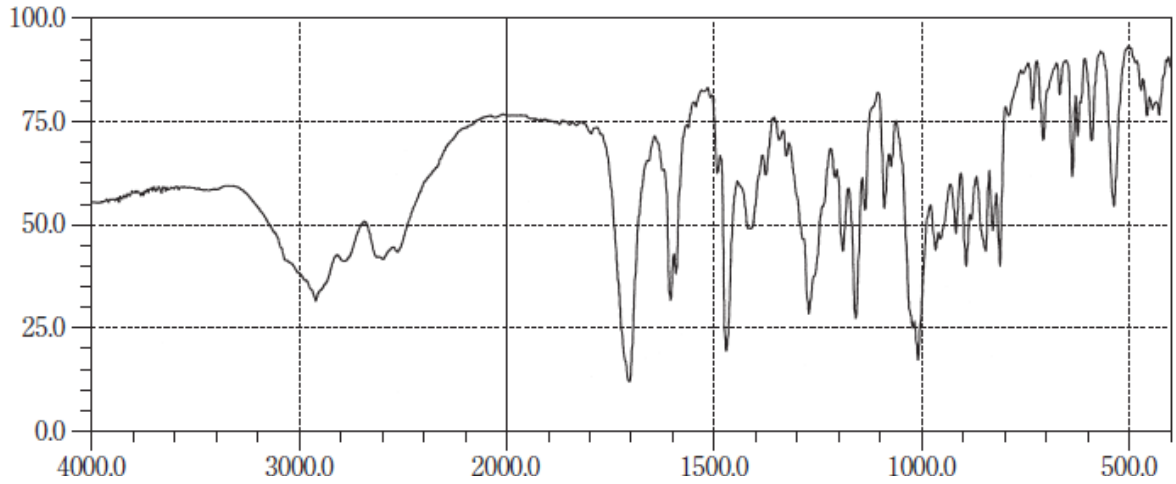
Simvastatin



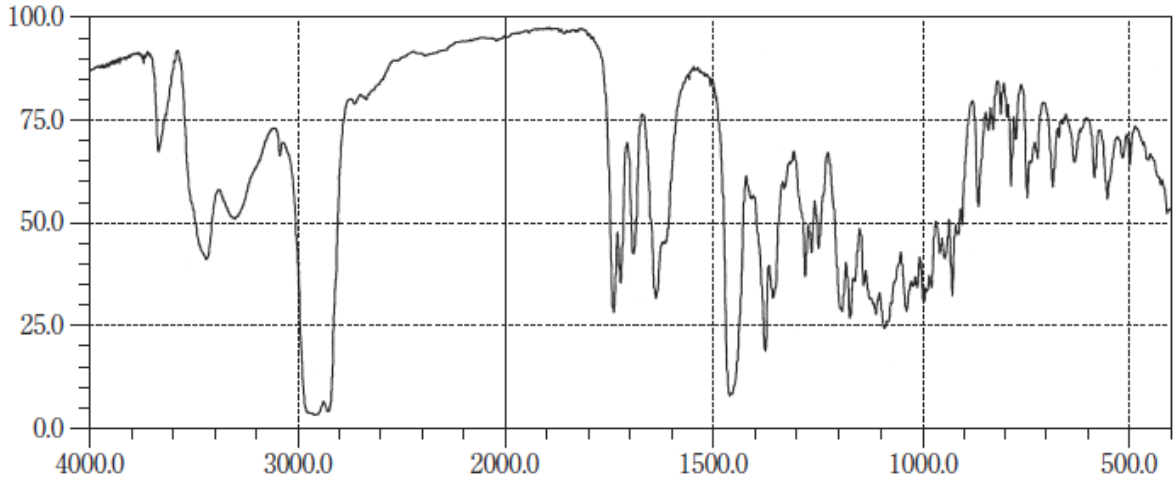
Purified Sodium Hyaluronate



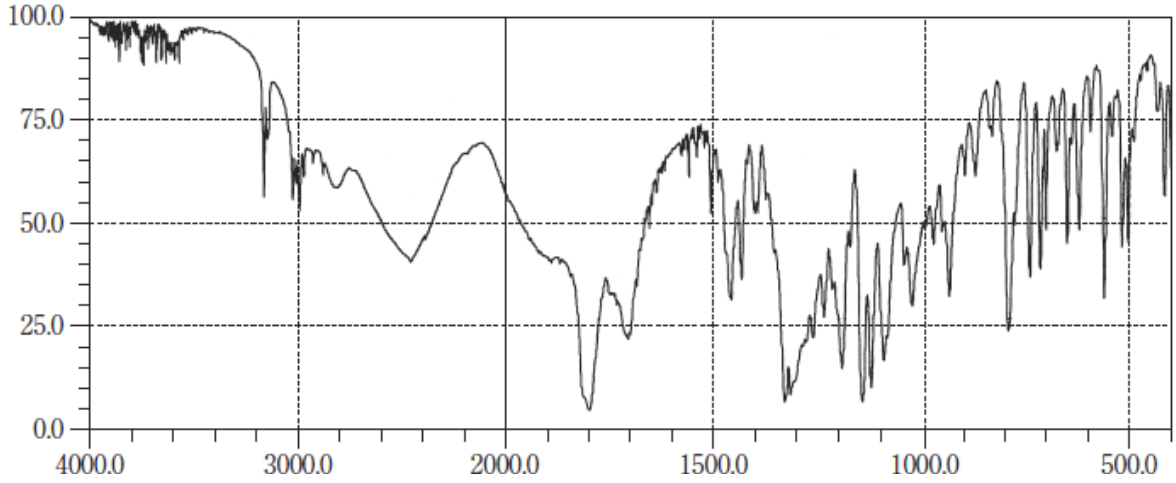
Sulindac



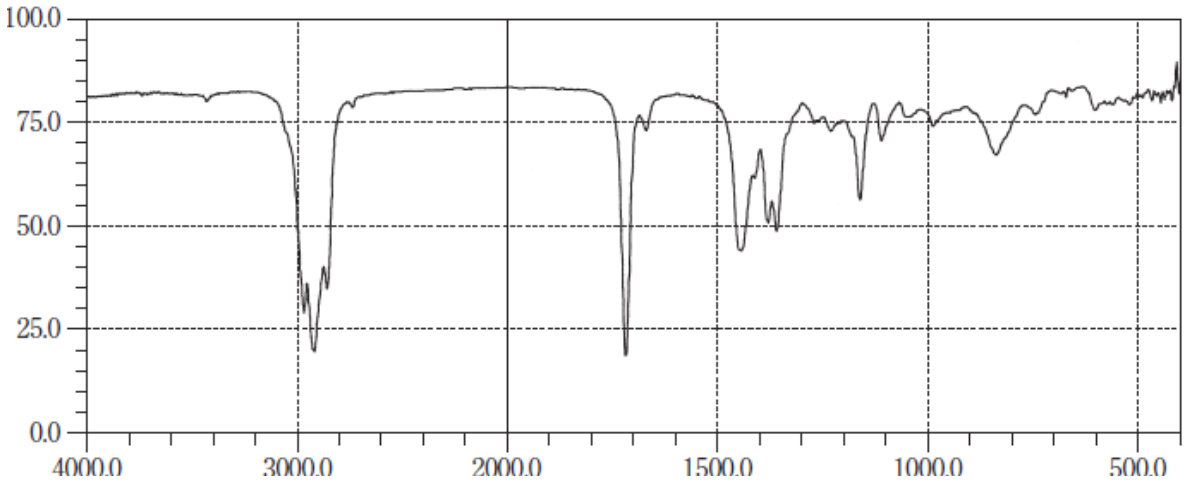
Tacrolimus Hydrate



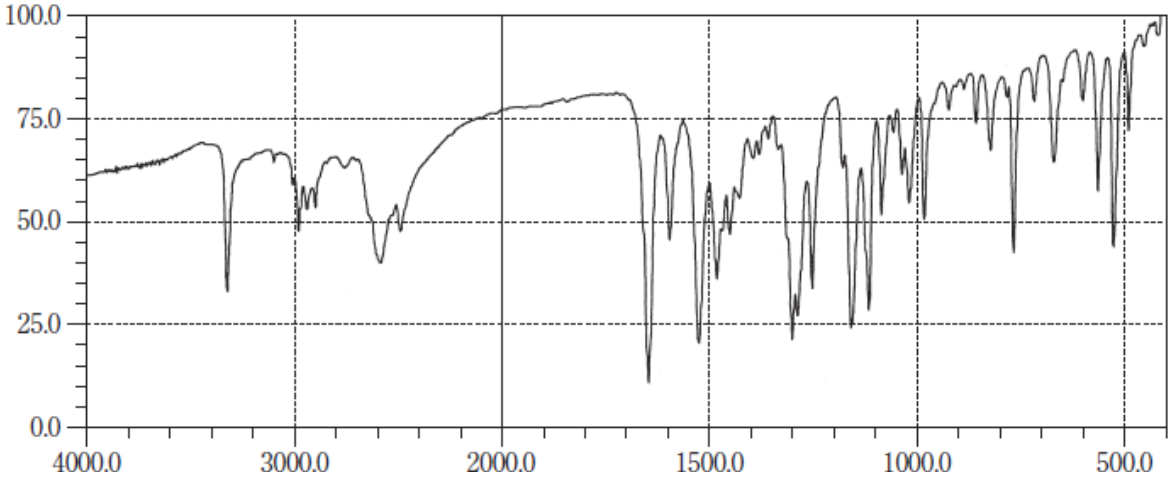
Tazobactam



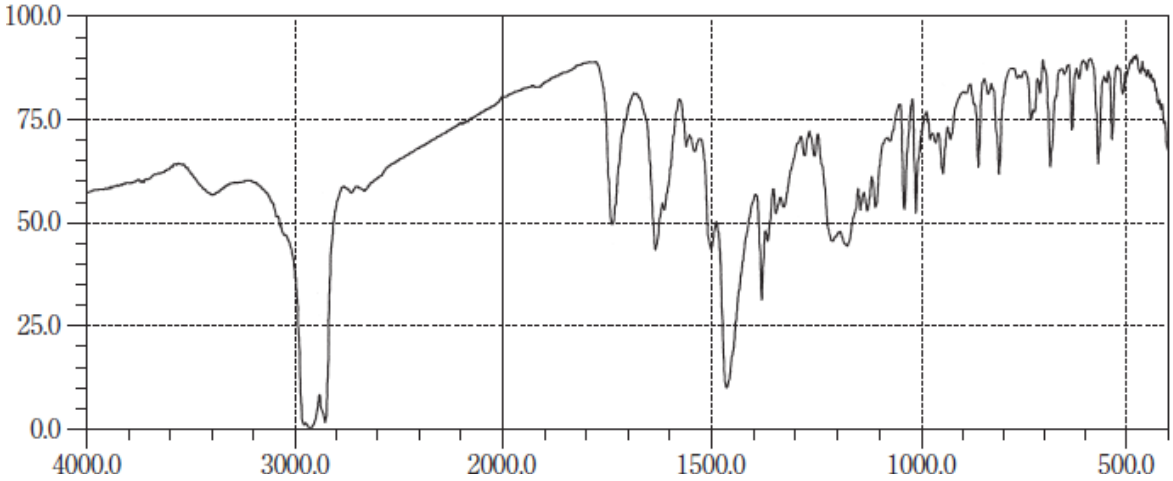
Teprenone



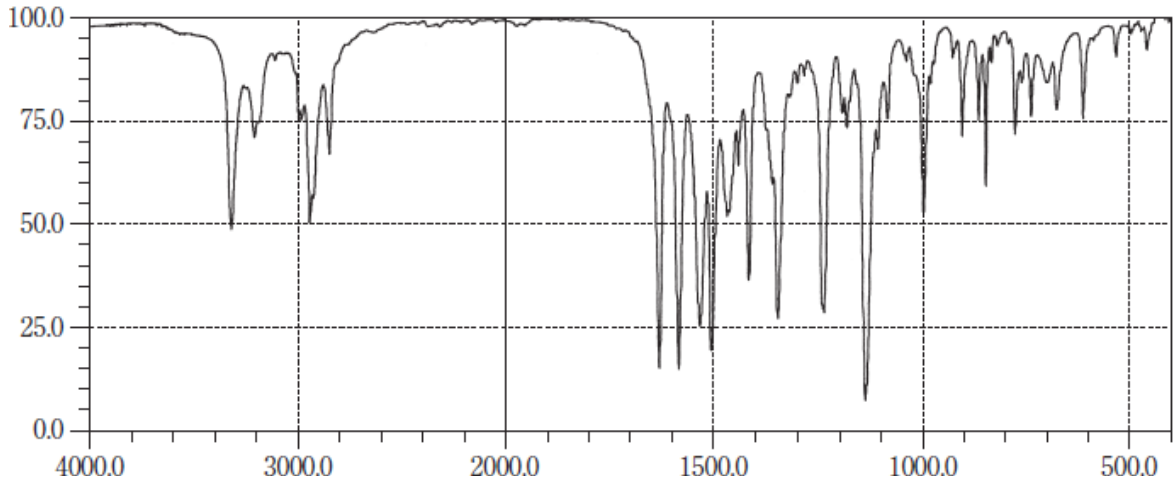
Tiapride Hydrochloride



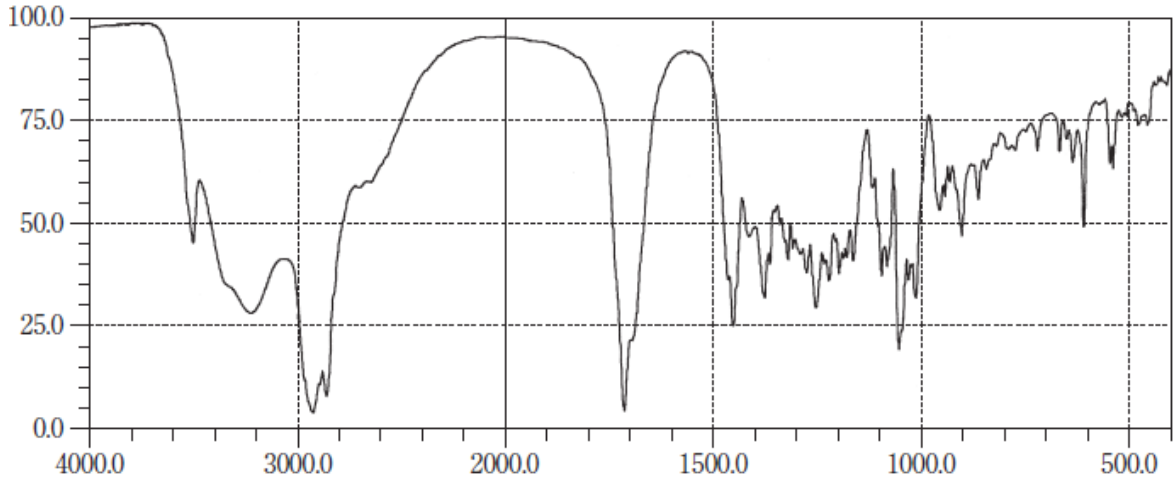
Tosufloxacin Tosilate Hydrate



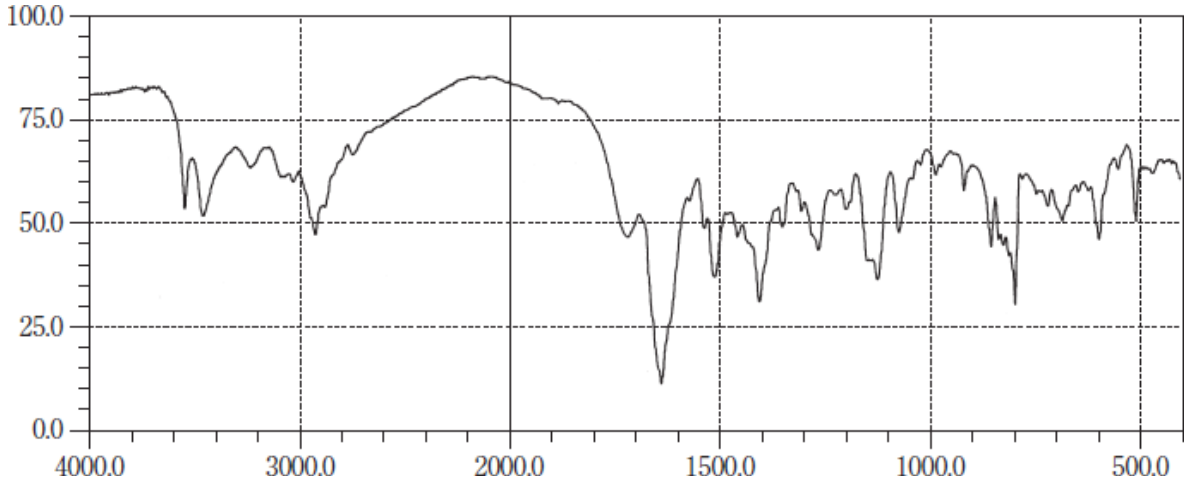
Troxipide



Ursodeoxycholic Acid



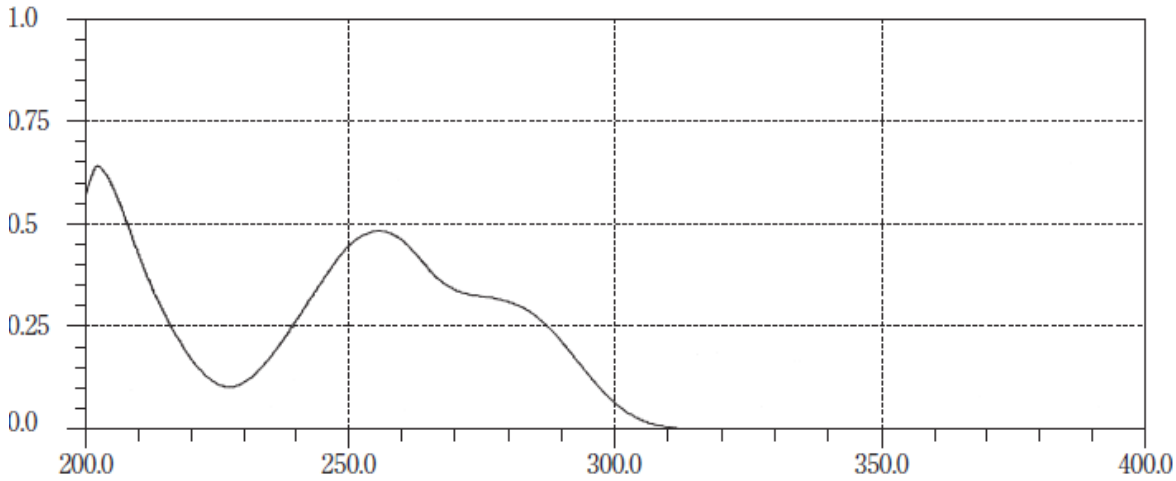
Zolpidem Tartrate



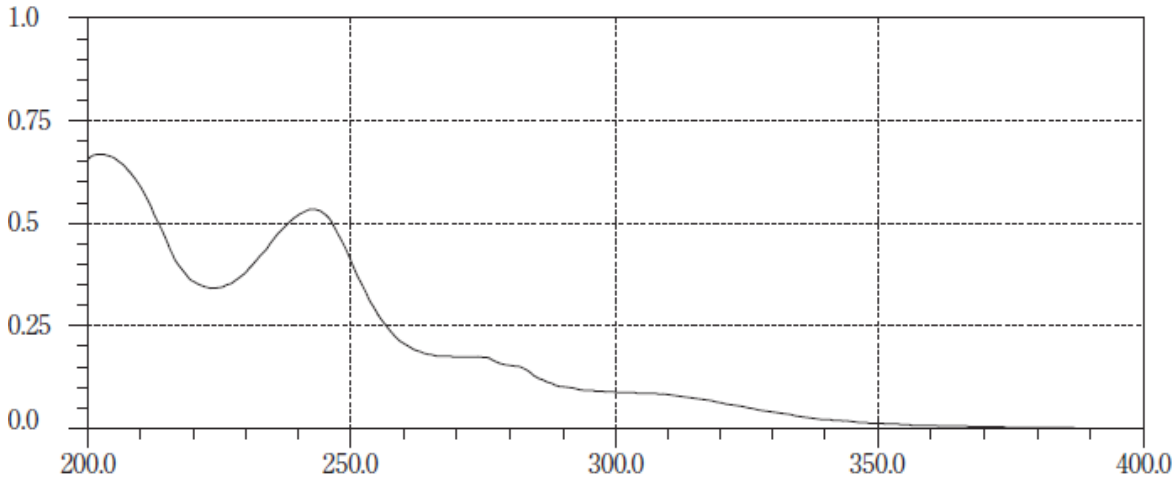
Ultraviolet-visible Reference Spectra

Add the following 41 spectra:

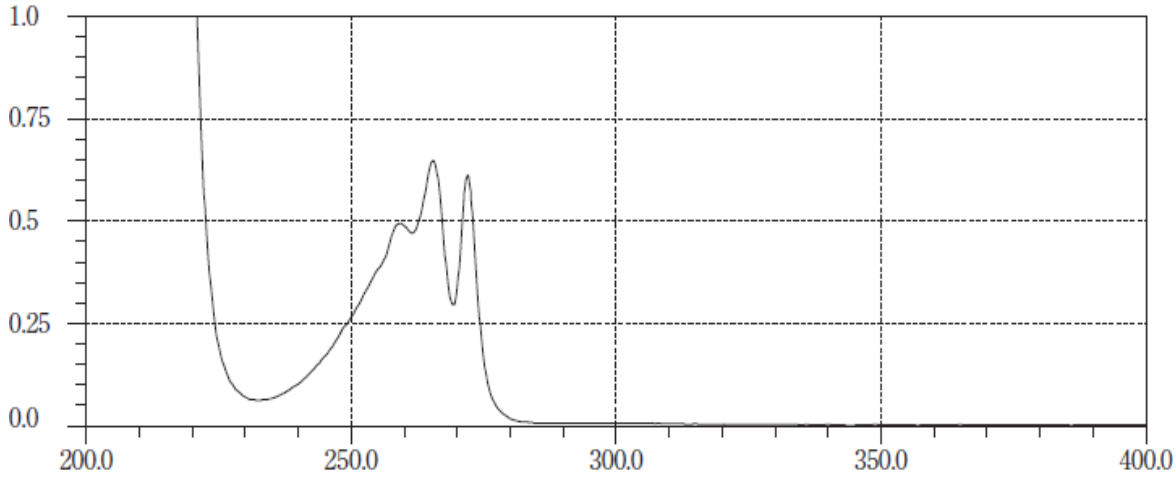
Aciclovir



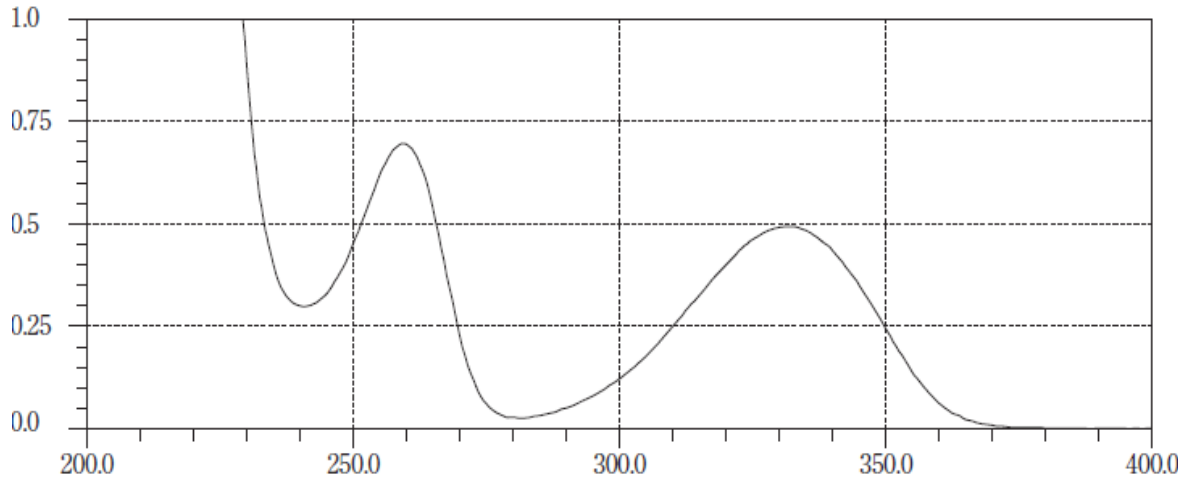
Amiodarone Hydrochloride



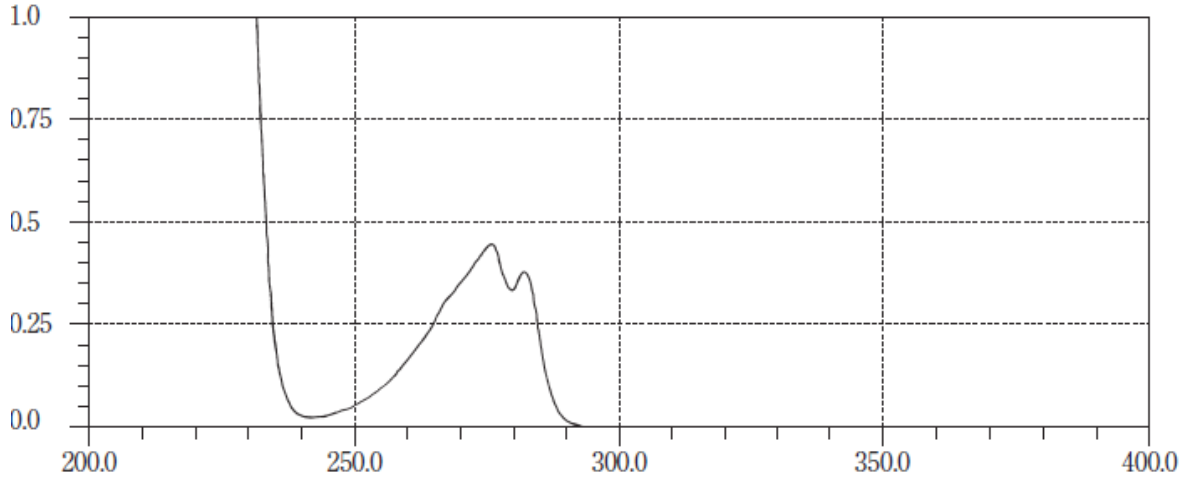
Aprindine Hydrochloride



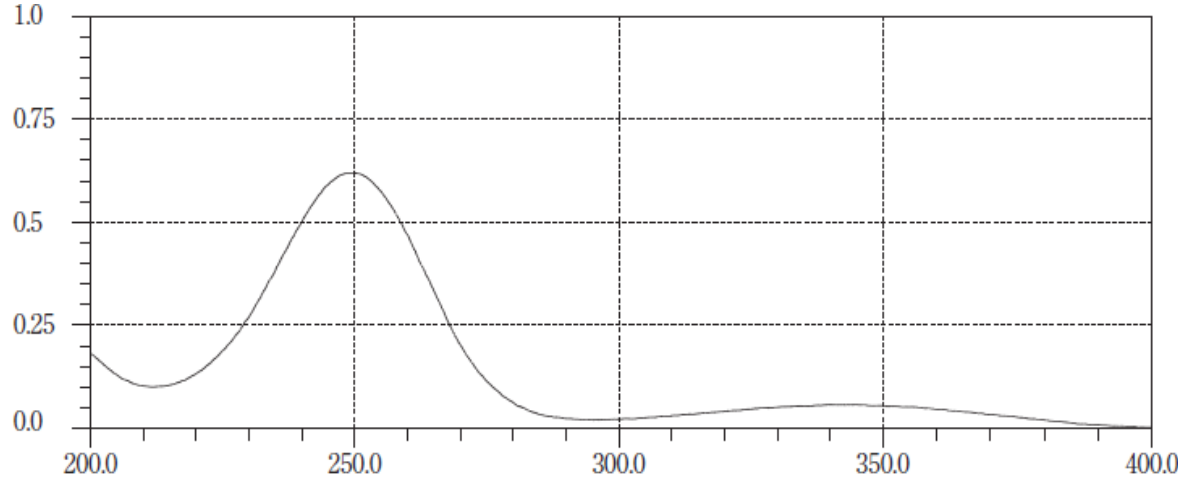
Argatroban Hydrate



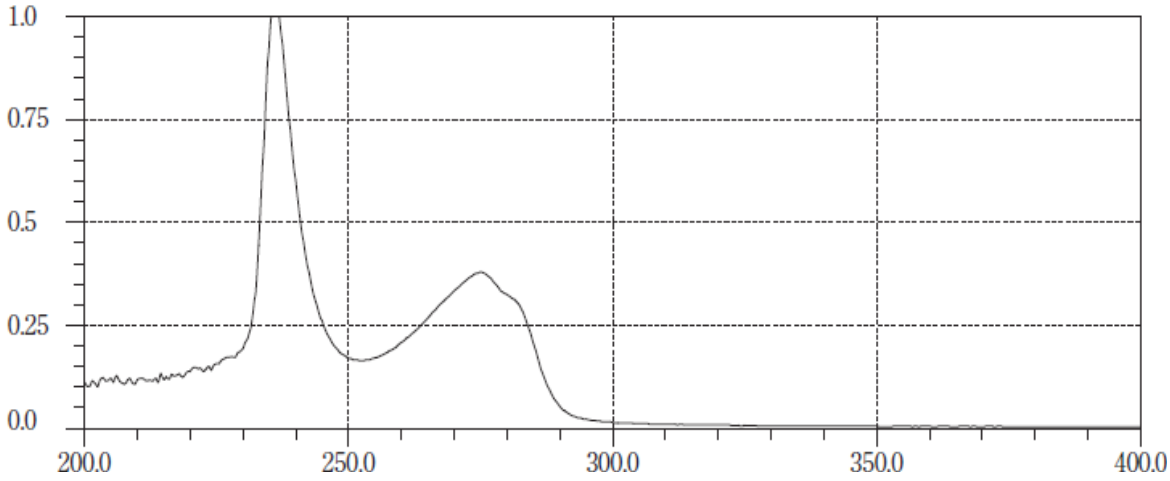
Betaxolol Hydrochloride



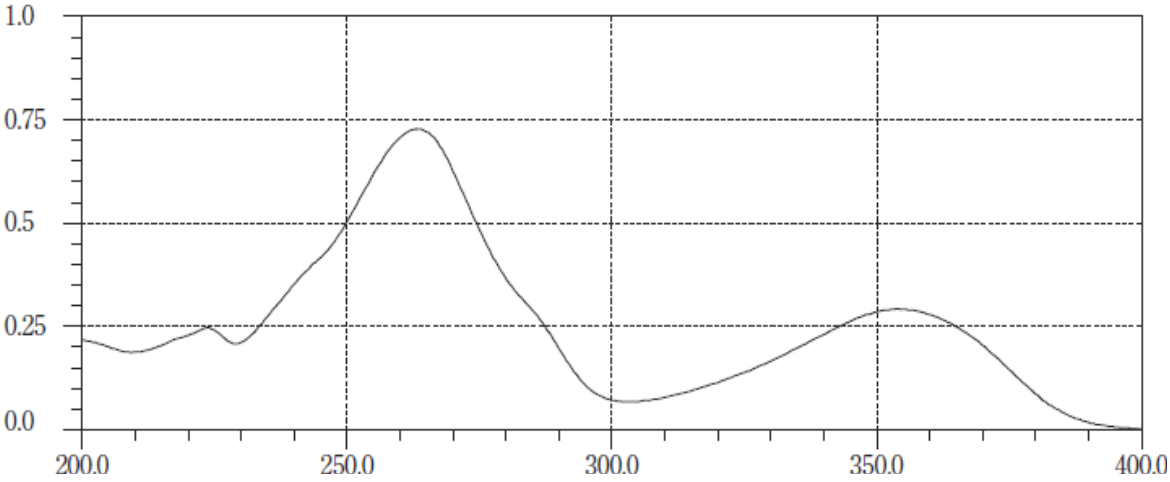
Cadralazine



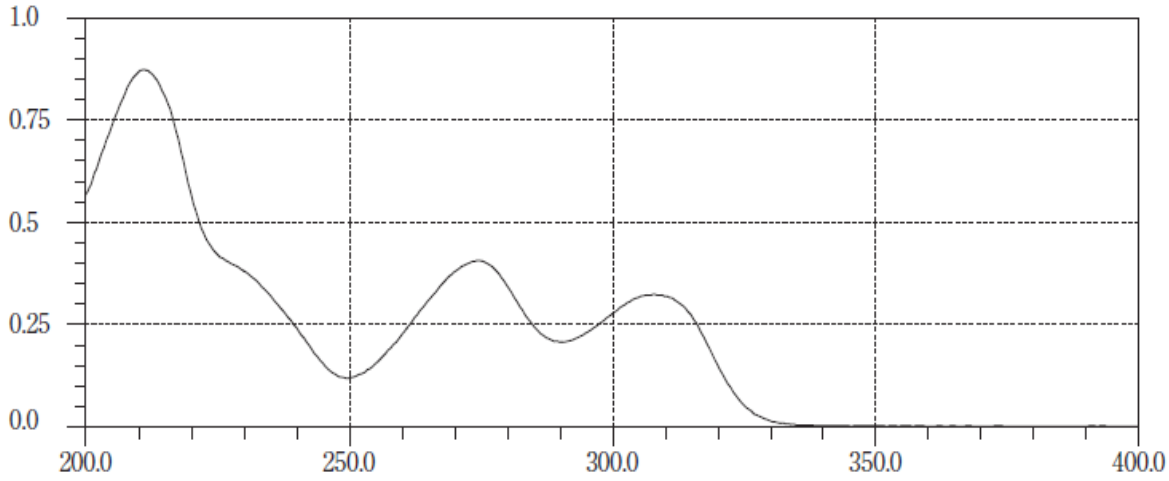
Calcitonin（Salmon）



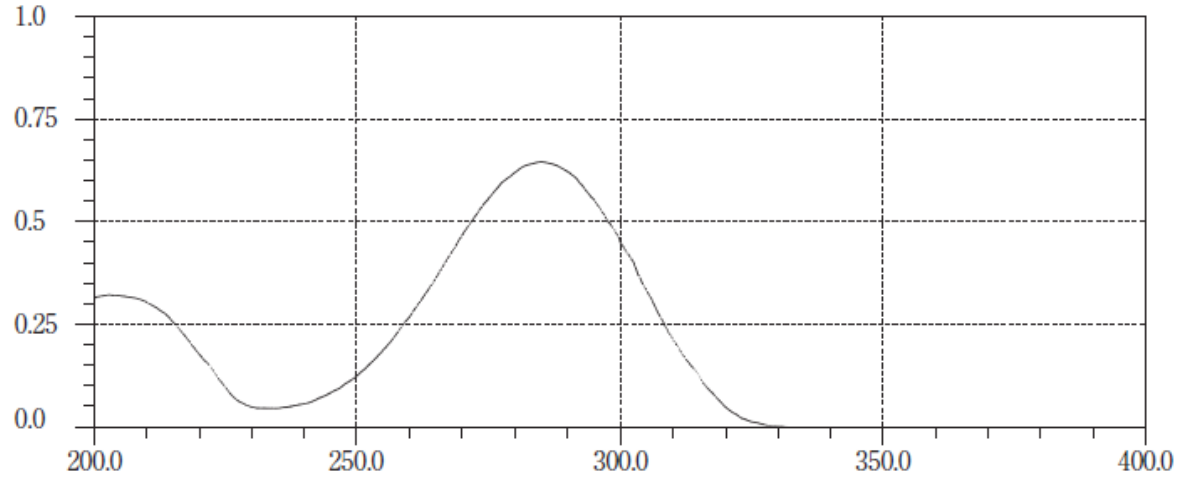
Cinoxacin



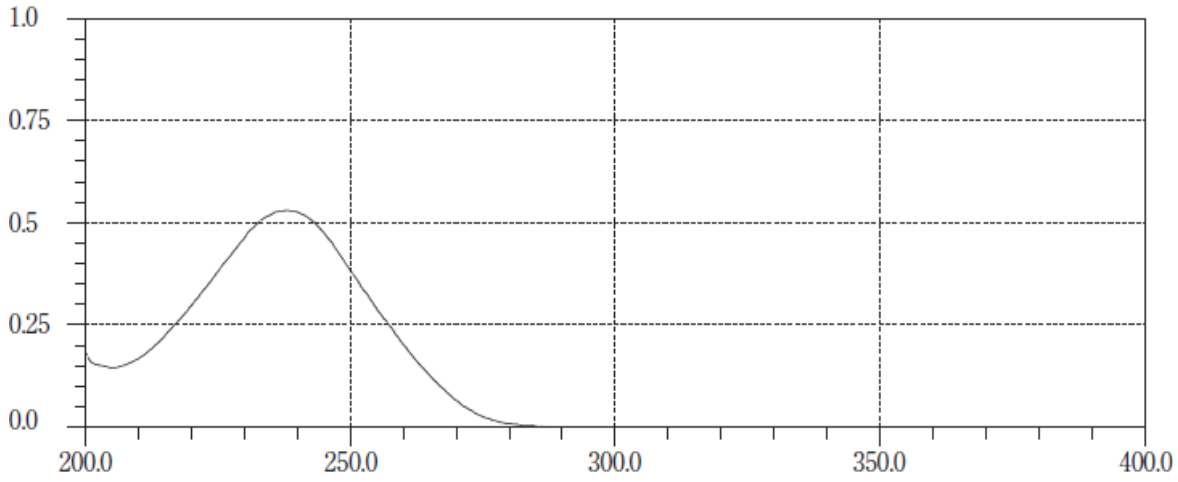
Clebopride Malate



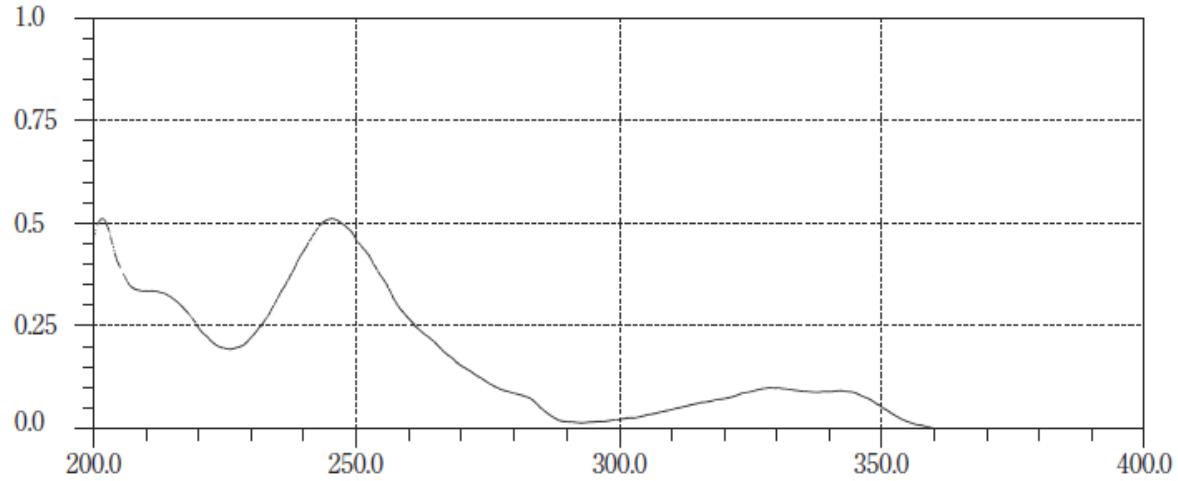
Danazol



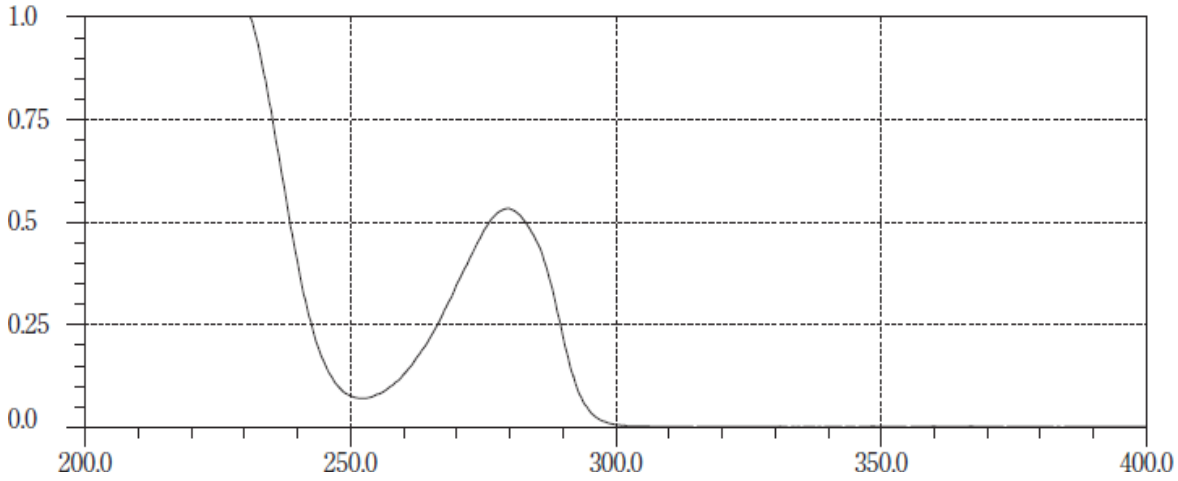
Diflucortolone Valerate



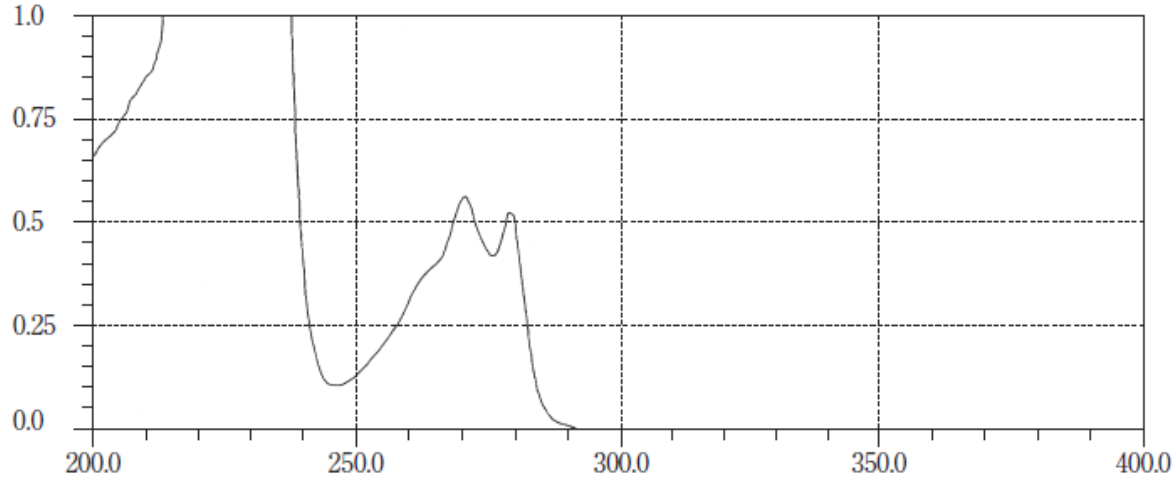
Doxazosin Mesilate



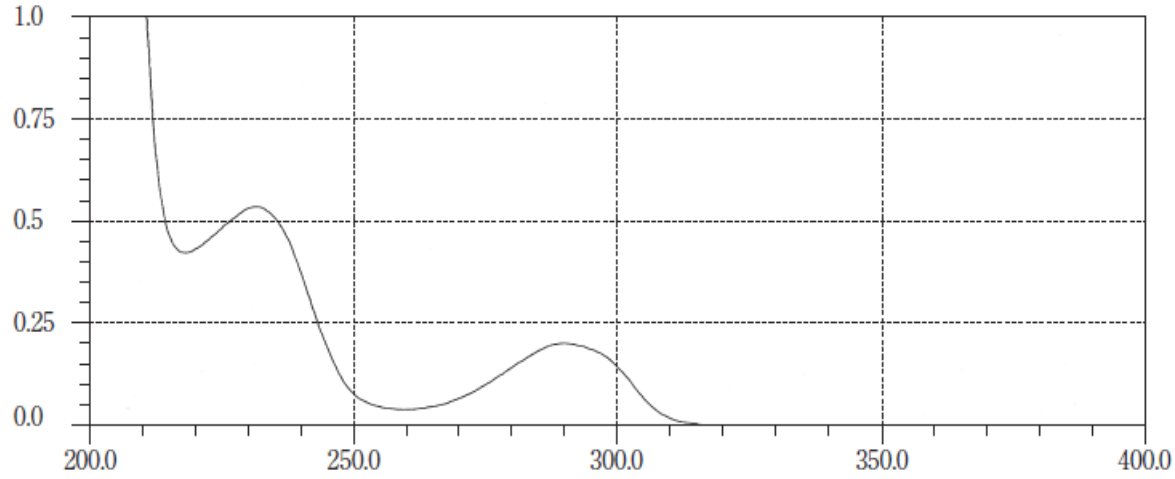
Droxidopa



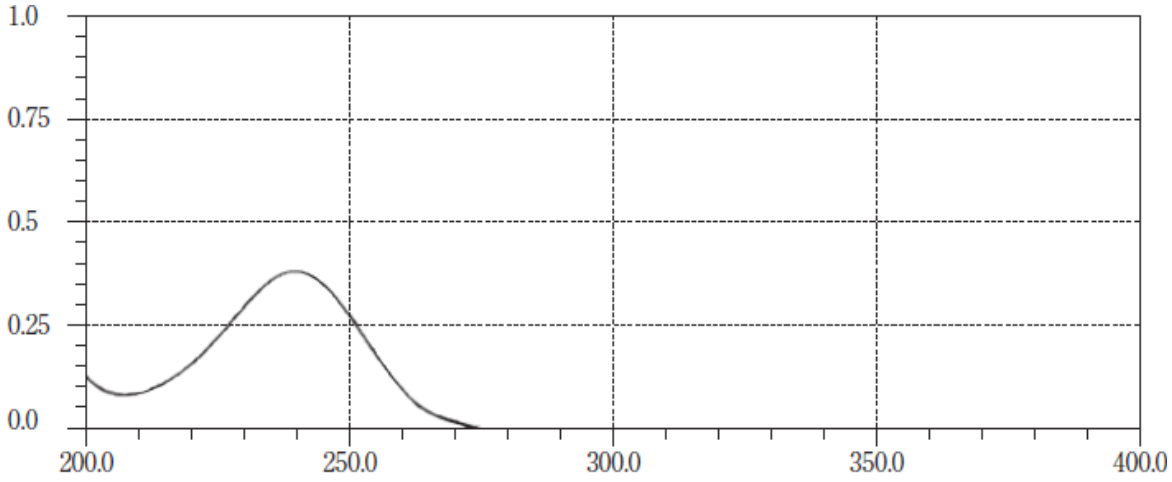
Ecabet Sodium Hydrate



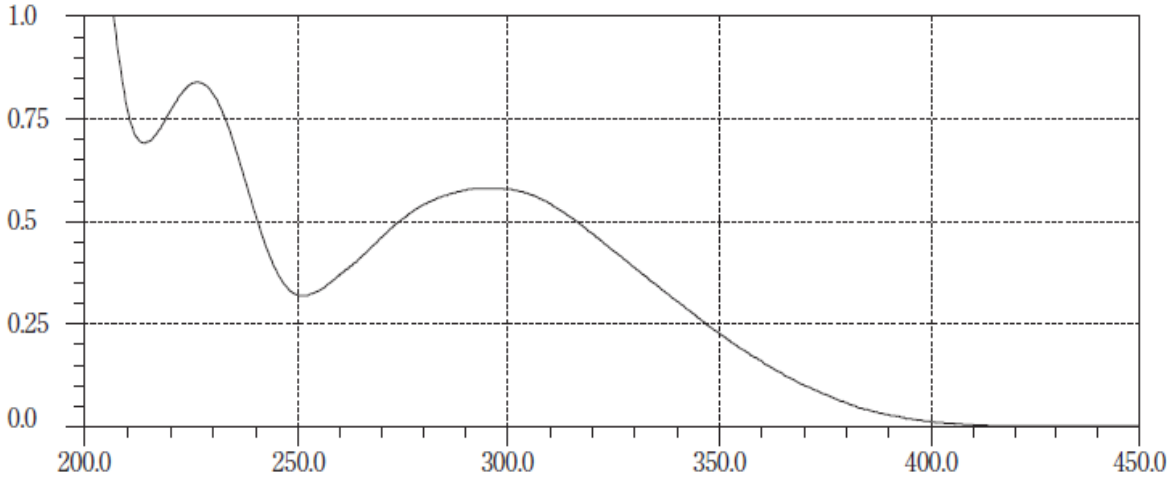
Ethenzamide



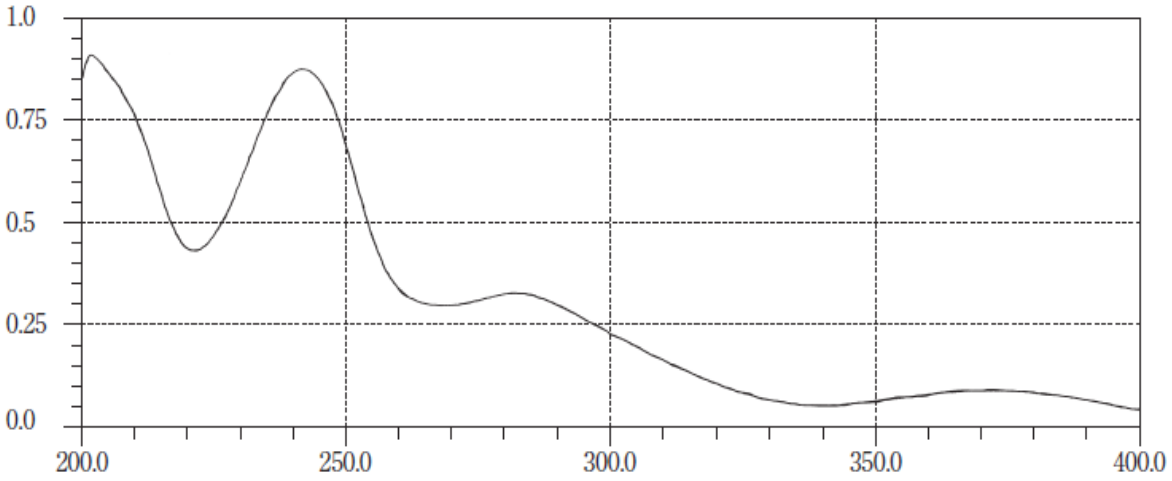
Fludrocortisone Acetate



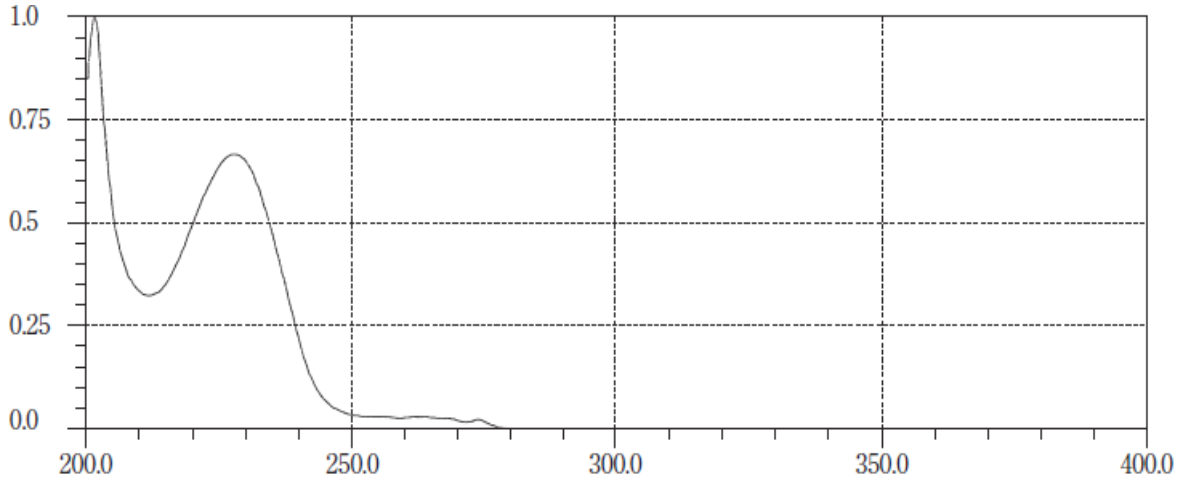
Flutamide



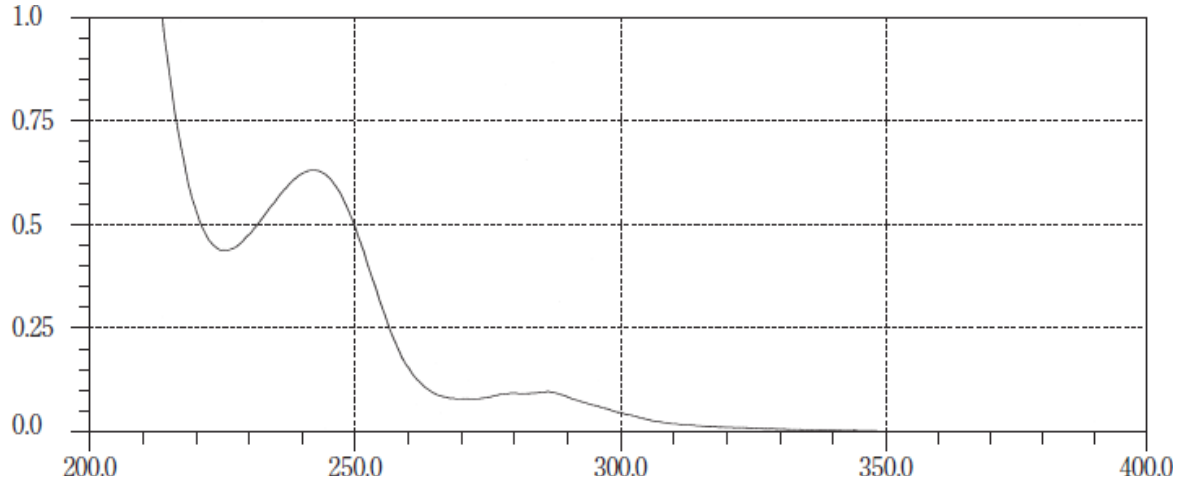
Flutoprazepam



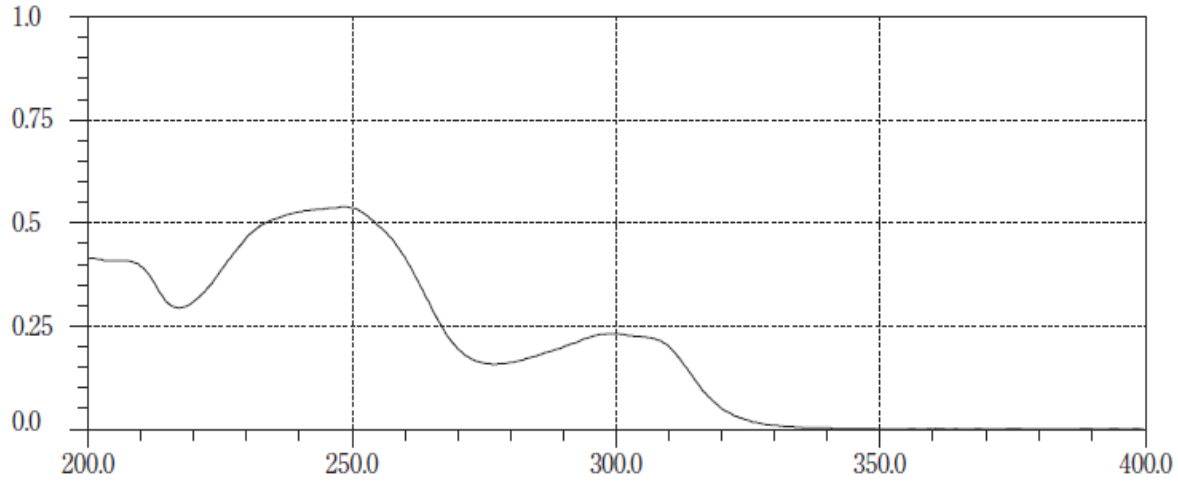
Gliclazide



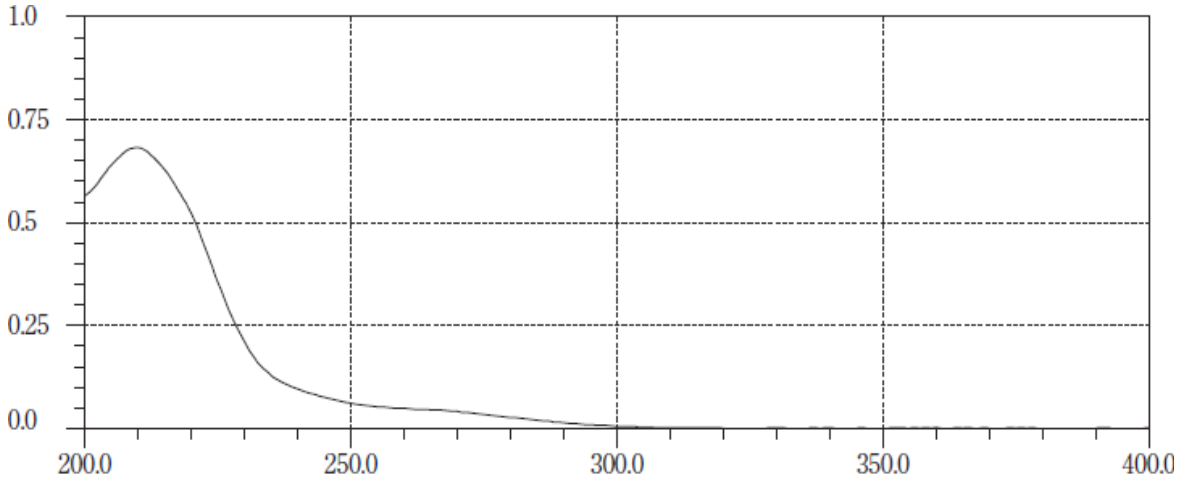
Indapamide



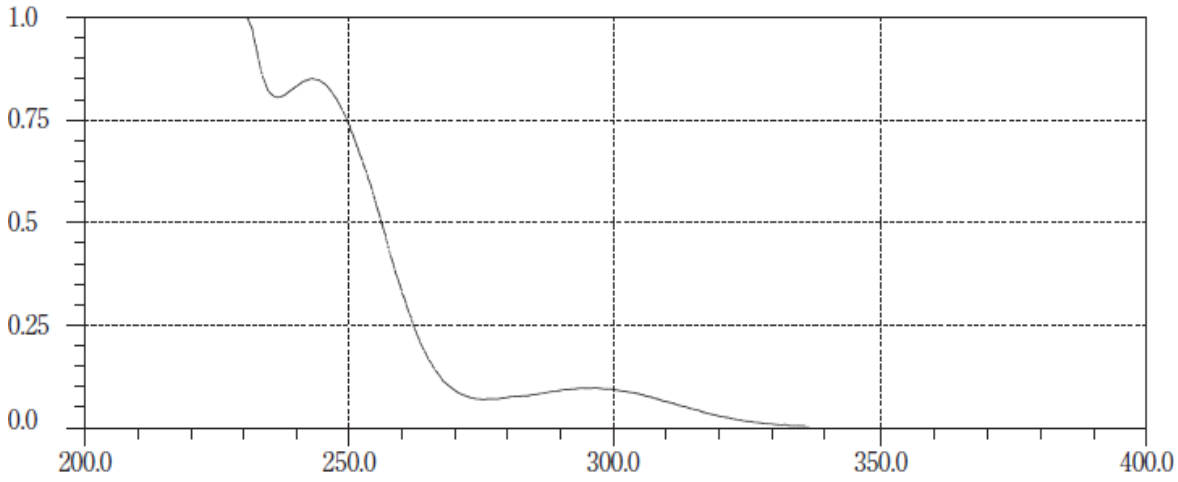
Ipriflavone



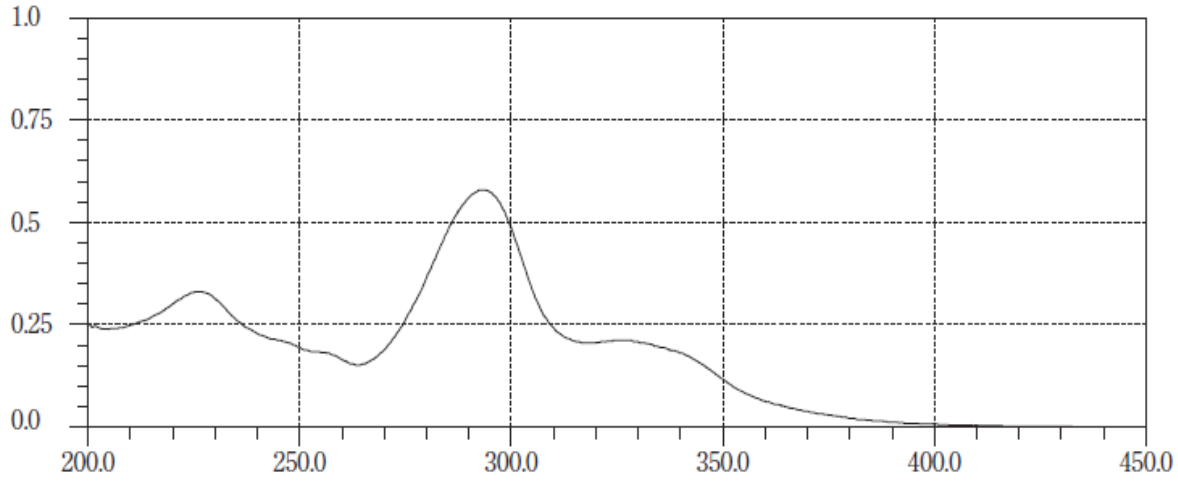
Irsogladine Maleate



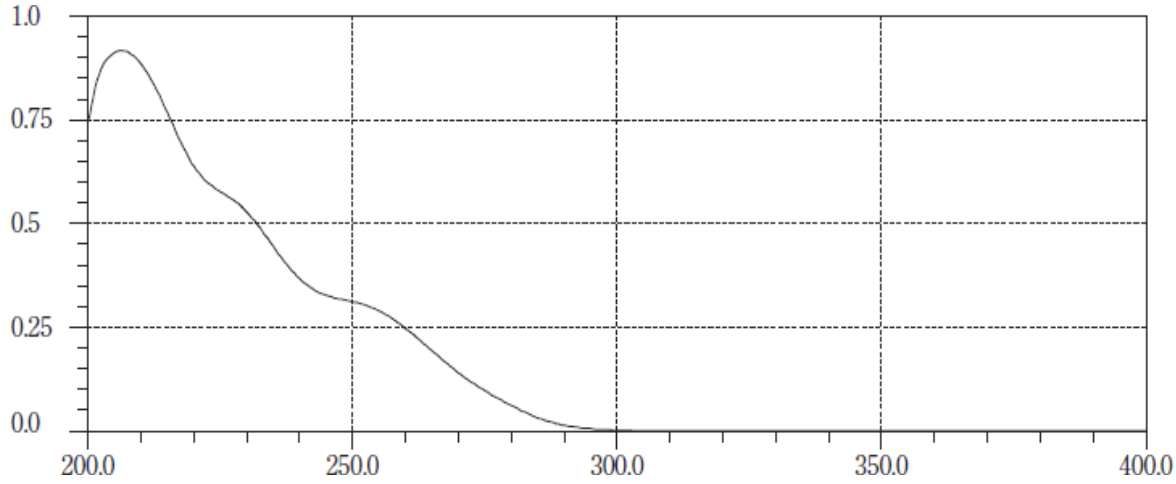
Ketoconazole



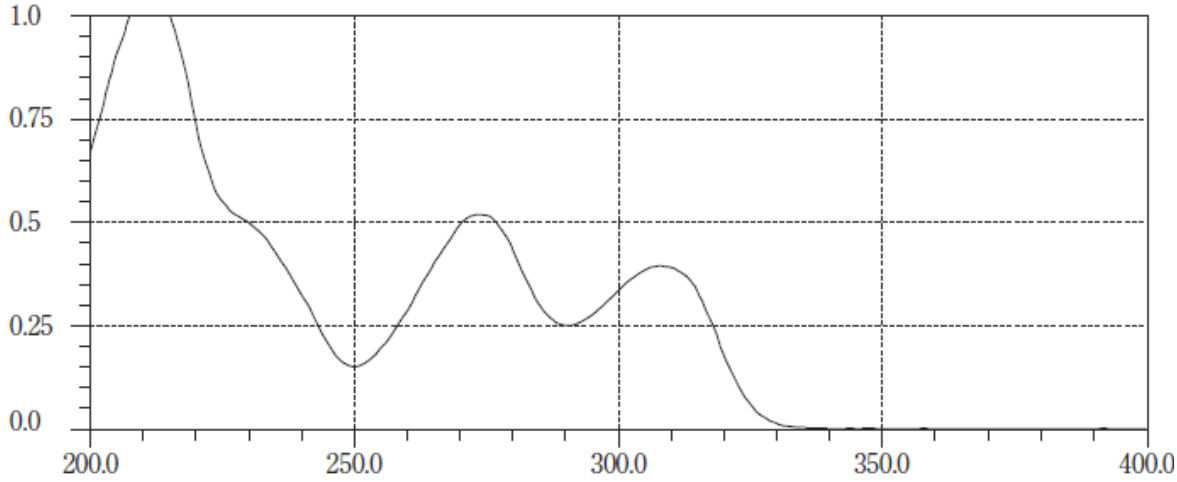
Levofloxacin Hydrate



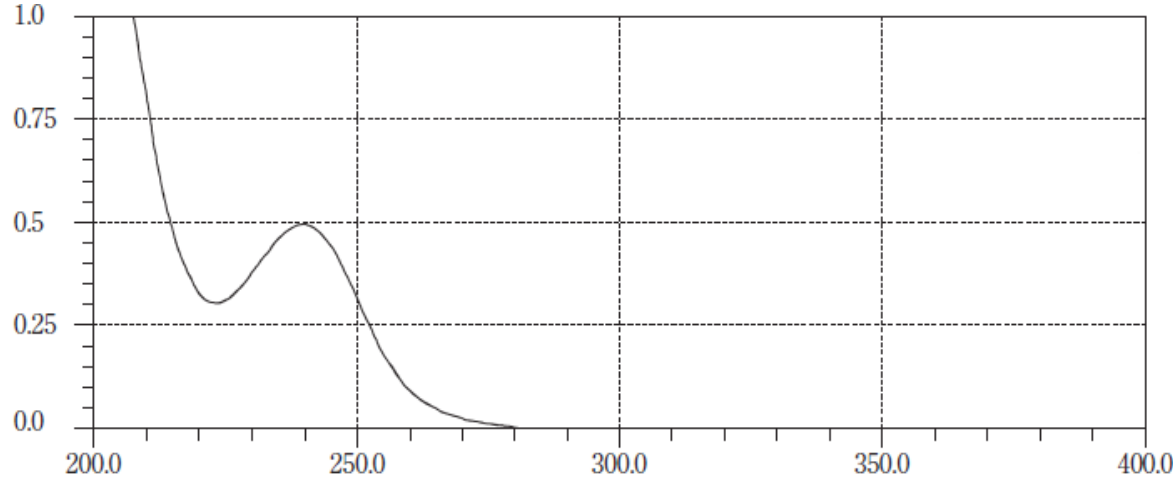
Losartan Potassium



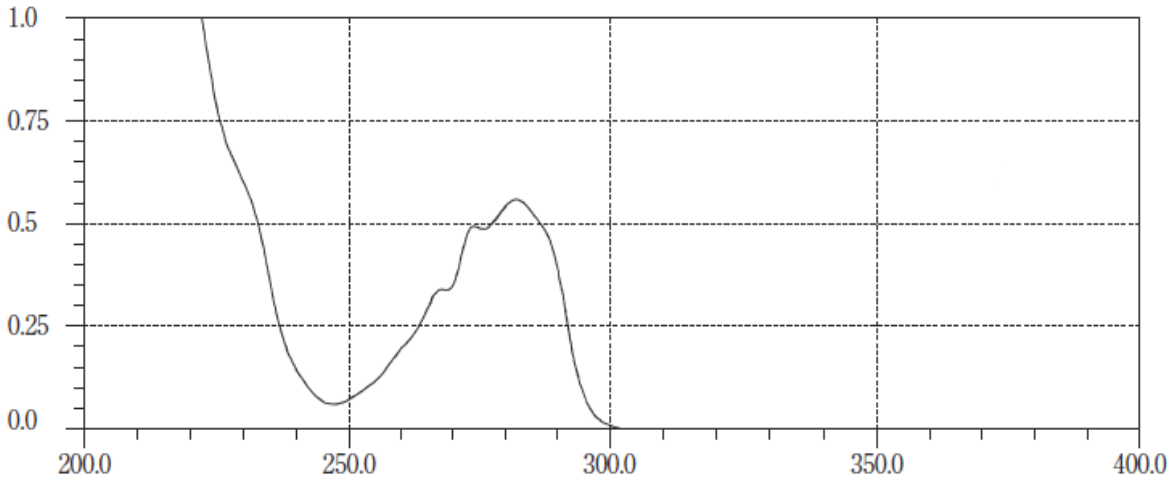
Mosapride Citrate Hydrate



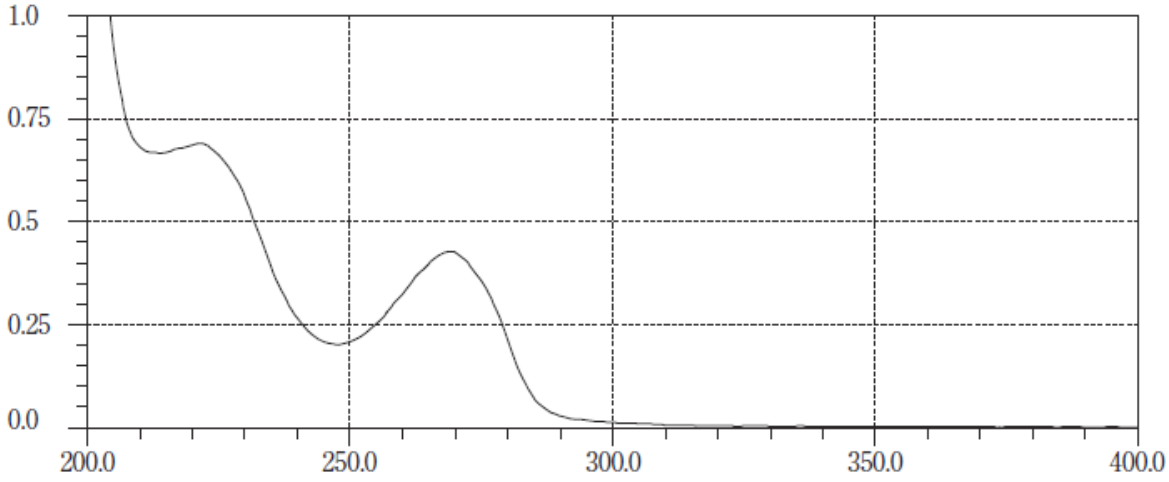
Phenobarbital



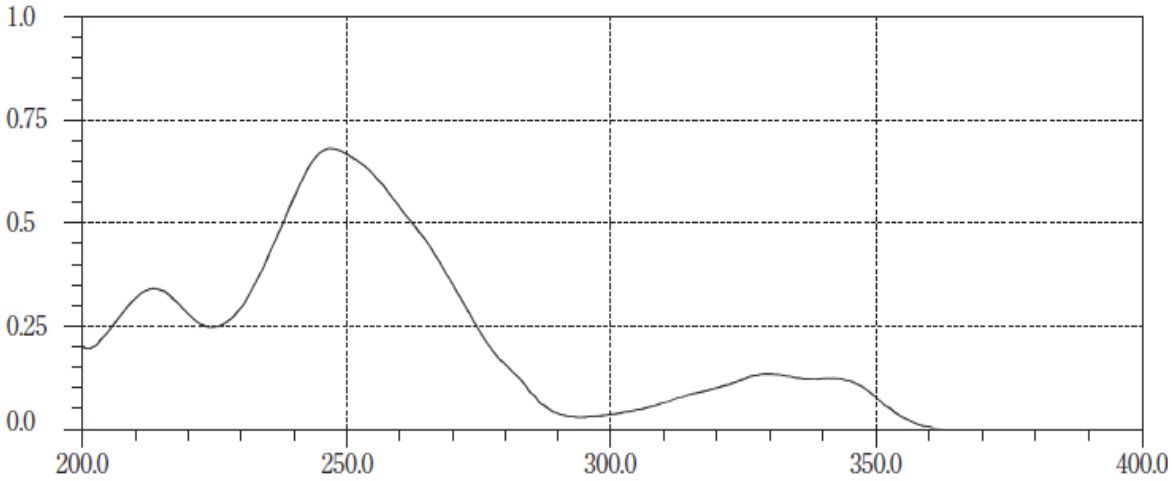
Pimozide



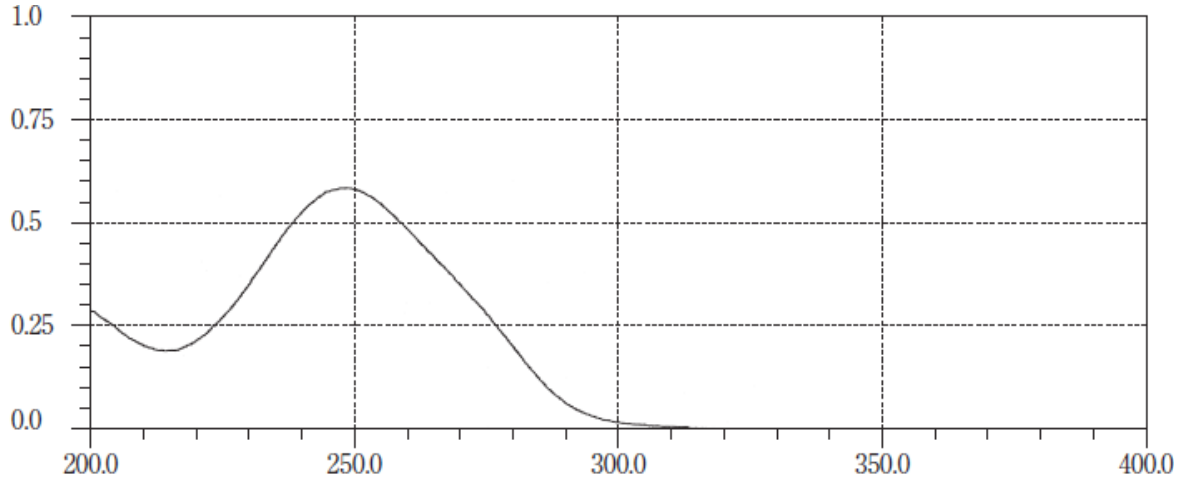
Pioglitazone Hydrochloride



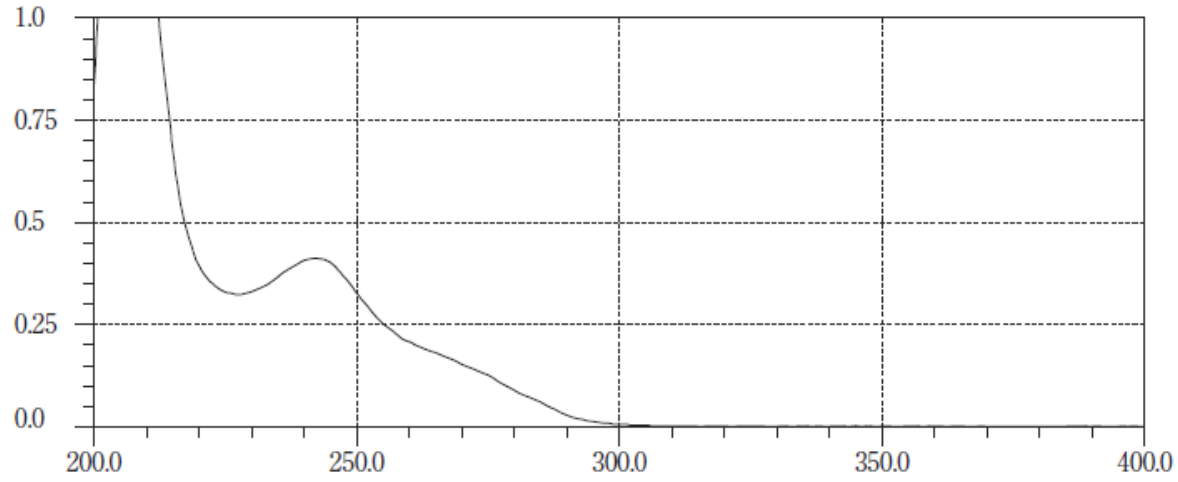
Prazosin Hydrochloride



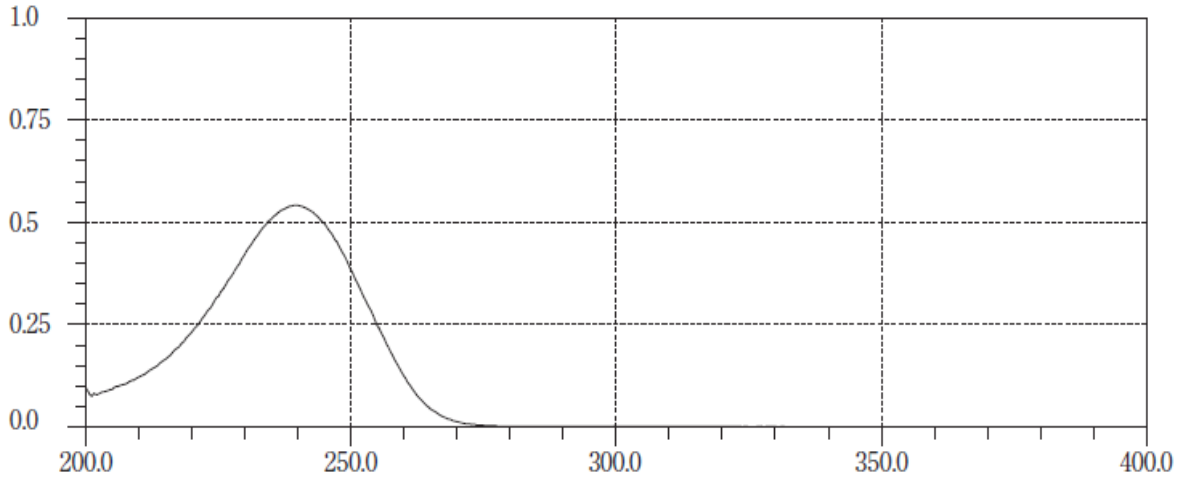
Prednisolone Sodium Phosphate



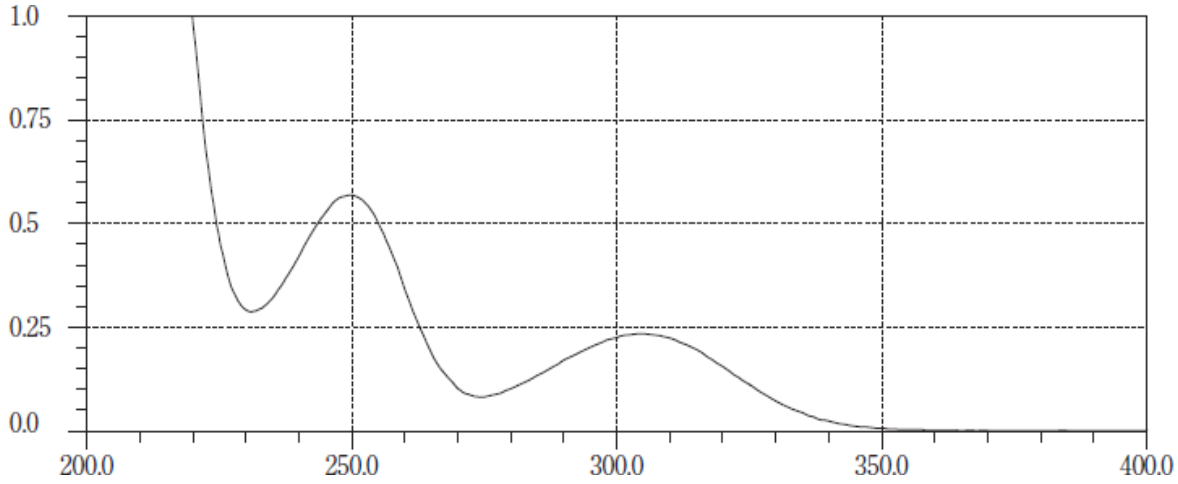
Probucol



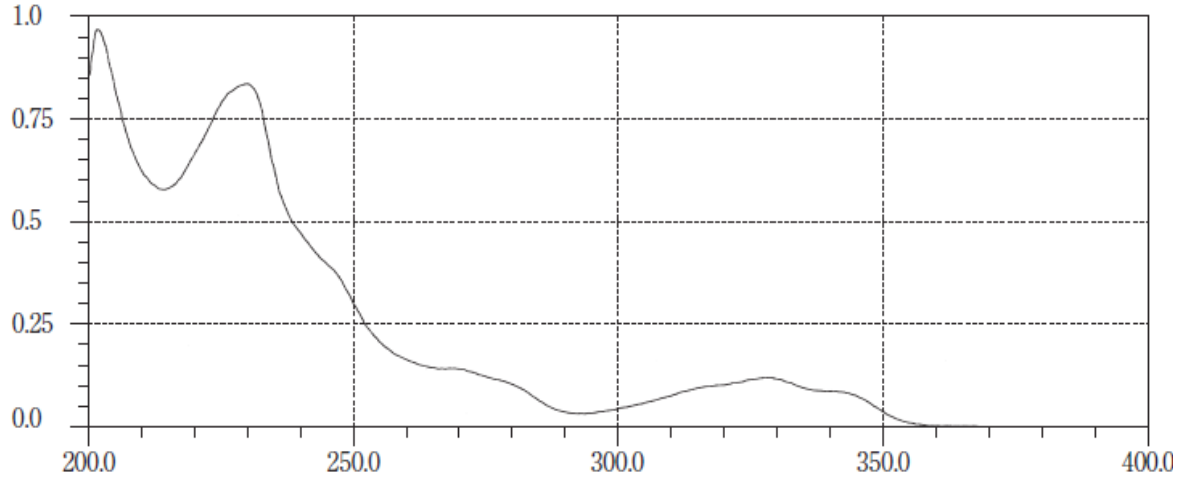
Progesterone



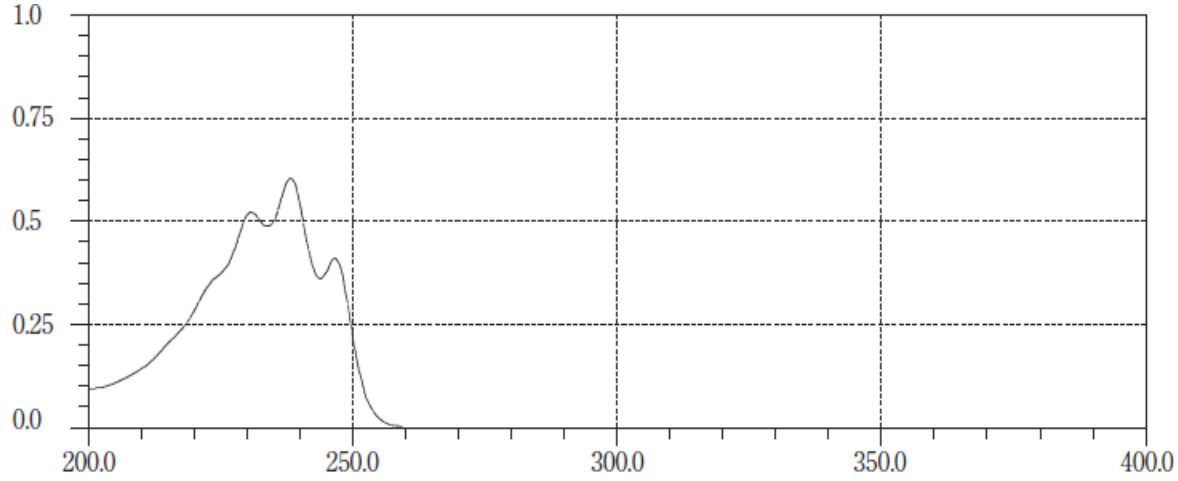
Propafenone Hydrochloride



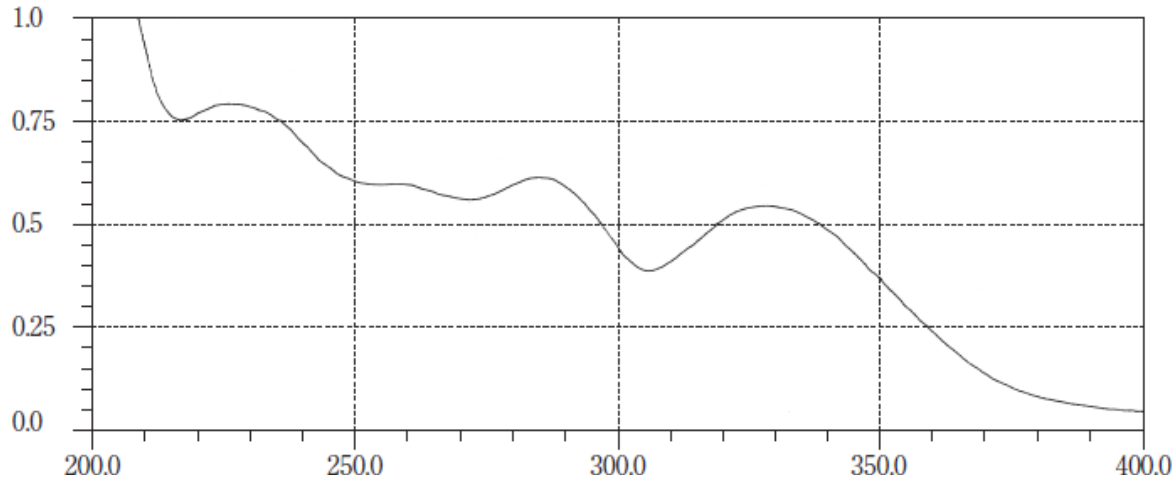
Rebamipide



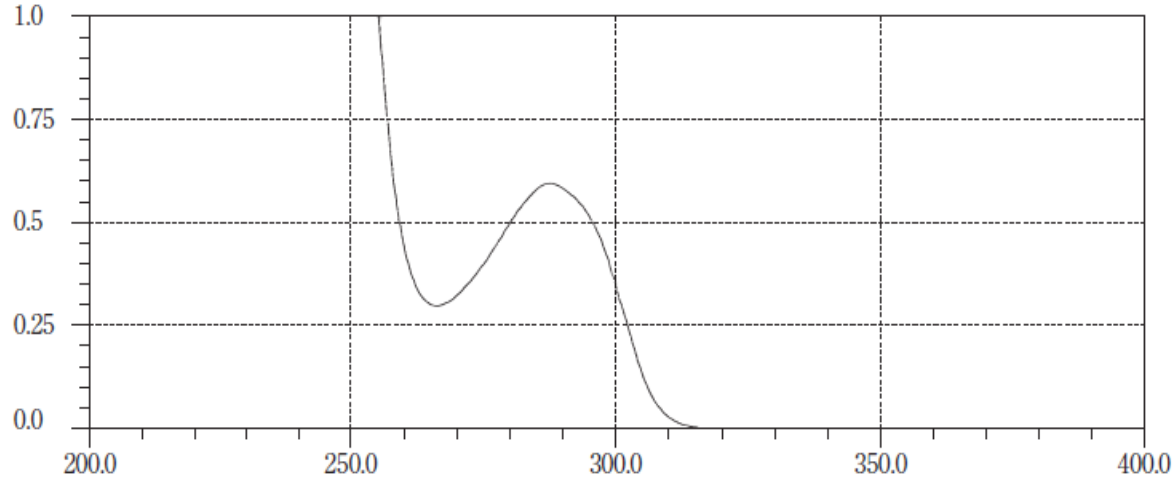
Simvastatin



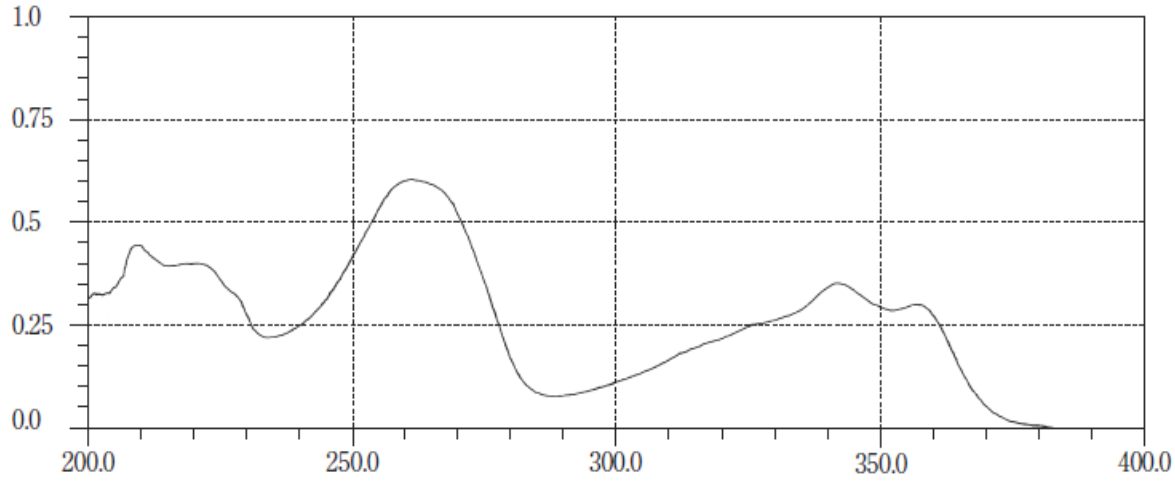
Sulindac



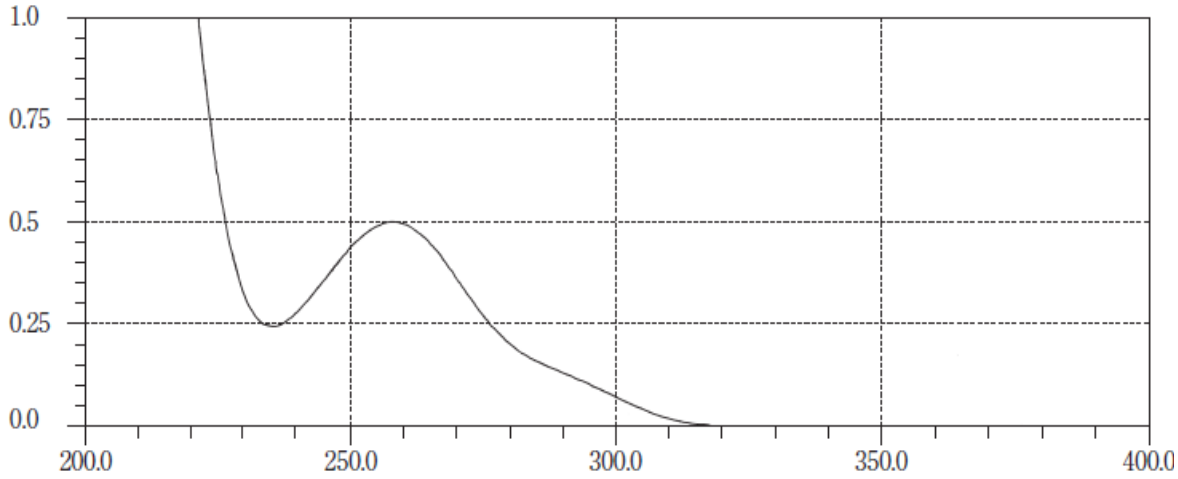
Tiapride Hydrochloride



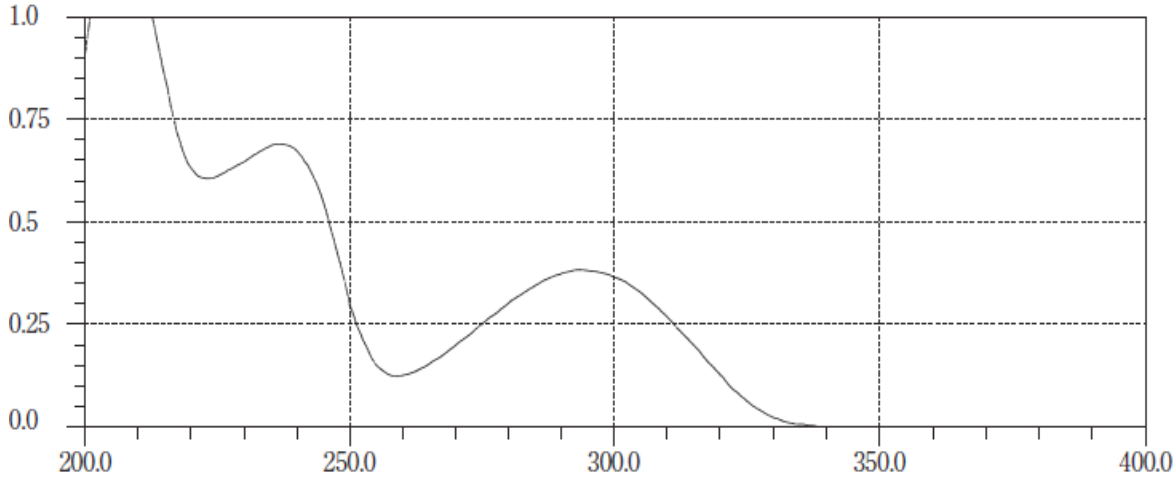
Tosufloxacin Tosilate Hydrate



Troxipide



Zolpidem Tartrate



General Information

GENERAL INFORMATION

8. International Harmonization Implemented in the Japanese Pharmacopoeia Fifteenth Edition

Add the following:

June 2008 (Rev.1)

Harmonized items	JP15 (Supplement II)	Remarks
Bulk Density and Tapped Density of Powders	**3.01 Determination of Bulk and Tapped Densities**	
	(Introduction)	JP's particular description: Explanation of this test method.
Bulk density	Bulk density	
Method 1: Measurement in a graduated cylinder	Method 1: Measurement in a Graduated Cylinder	
Procedure	Procedure	
Method 2: Measurement in a volumeter	Method 2: Measurement in a Volumeter	
Apparatus	Apparatus	
Procedure	Procedure	
Method 3: Measurement in a vessel	Method 3: Measurement in a Vessel	
Apparatus	Apparatus	
Procedure	Procedure	
Tapped density	Tapped density	
Method 1	Method 1	
Apparatus	Apparatus	
Procedure	Procedure	
Method 2	Method 2	
Procedure	Procedure	
Method 3	Method 3	
Procedure	Procedure	
Measures of powder compressibility	Measures of powder compressibility	

May 2007

Harmonized items	JP15 (Supplement II)	Remarks
Gas Pycnometric Density of Solids	**3.03 Powder Particle Density Determination**	.
(Introduction)	(Introduction)	JP's particular description: application of this test method
Apparatus	1. Apparatus	
	Calibration of apparatus	The range of temperature during the measurement is described in the Procedure.
Method	2. Procedure	
Expression of the results	2. Procedure	

October 2006

Harmonized items	JP15 (Supplement II)	Remarks
Rice Starch	**Rice Starch**	
Definition	Definition	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	

May 2007

Harmonized items	JP15 (Supplement II)	Remarks
	GENERAL INFORMATION	
Powder Fineness	Powder Fineness	

Change to read:

14. Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products

This document describes the currently available methods of mycoplasma testing that should be performed for cell substrates that are used in the manufacture of biotechnological/biological products.

Methods suggested for detection of mycoplasma are, A. culture method, B. indicator cell culture method, and C. polymerase chain reaction (PCR) method.

Mycoplasma testing should be performed on the master cell bank (MCB) and the working cell bank (WCB), as well as on the cell cultures used during the manufacturing process of the product. For the assessment of these cells, mycoplasma testing should be performed using both methods A and B. Method B, however, does not detect only DNA derived from mycoplasma. Therefore, if a positive result is obtained only from method B, method C can be used to determine whether mycoplasma is actually present. When method C is used, it is necessary to demonstrate the rationale for determining a negative result. In such a case, the sensitivity and specificity of the method, the appropriateness of the sample preparation, and the suitability of the selection of the test method, including selection of reagents, reaction conditions and primers should be taken into account.

Prior to mycoplasma testing, the sample should be tested to detect the presence of any factors inhibiting the growth of mycoplasma. If such growth-inhibiting factors are detected they should be neutralized or eliminated by an appropriate method, such as centrifugation or cell passage.

If the test will be performed within 24 hours of obtaining the sample, the sample should be stored at a temperature between 2ºC and 8ºC. If more than 24 hours will elapse before the test is performed, the sample should be stored at –60ºC or lower.

If mycoplasma is detected, additional testing to identify the species may be helpful in determining the source of contamination.

A. Culture Method

1. Culture Medium

Both agar plates and broth are used. Each lot of agar and broth medium should be free of antibiotics except for penicillin. Refer to the Minimum Requirements for Biological Products regarding selection of the culture media. Other culture media may be used if they fulfill the requirements described in the following section 2.

2. Suitability of Culture Medium

Each lot of medium should be examined for mycoplasma growth-promoting properties. To demonstrate the capacity of the media to detect known mycoplasma, each test should include control cultures of at least two known species or strains of mycoplasma, one of which should be a dextrose fermenter (i.e., *M. pneumoniae* ATCC15531 or equivalent species or strains) and one of which should be an arginine hydrolyser (i.e., *M. orale* ATCC23714 or equivalent species or strains). The mycoplasma strains used for the positive control tests should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately. Inoculate the culture medium with 100 colony-forming units (CFU) or 100 color-changing units (CCU) or less.

3. Culture and Observation

1) Inoculate no less than 0.2 mL of test sample (cell suspension) in evenly distributed amounts over the surface of each of two or more agar plates. After the surfaces of the inoculated plates are dried, the plates should be incubated under microaerophilic conditions in an atmosphere of nitrogen containing 5 to 10 percent carbon dioxide and adequate humidity at 36 ± 1ºC for no less than 14 days.

2) Inoculate no less than 10 mL of the test sample (cell suspension) into each of one or more vessels containing 100 mL of broth medium, and incubate at 36 ± 1ºC.

If the culture medium for the sample cells contains any growth-inhibiting factors, such as antibiotics, these factors must be removed. A method such as centrifugation is recommended for this purpose. Refer to the Validation tests for growth-inhibiting factors described in the Minimum Requirements for Biological Products for the detection of growth-inhibiting factors.

3) Subculture 0.2 mL of broth culture from each vessel on the 3rd, 7th, and 14th days of incubation onto two or more agar plates. The plates should be incubated microaerophilically at 36 ± 1ºC for no less than 14 days.

4) Examination of all plates for mycoplasma colonies should be done microscopically on the 7th and 14th day at 100 times magnification or greater.

B. Indicator Cell Culture Method

Using Vero cell culture substrate, pretest the suitability of the method using an inoculum of 100 CFU or 100 CCU or less of *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains).

An equivalent indicator cell substrate and suitable mycoplasma strains may be acceptable if data demonstrate at least equal sensitivity for the detection of known mycoplasma contaminants. The mycoplasma strains should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately, and the unit of inoculation should be determined before use. The cell substrate used should be obtained from a qualified cell bank and certified to be mycoplasma free. The acquired cells should be carefully cultured and propagated, and sufficient volumes of seed stock should be prepared with the proper precautions to avoid mycoplasma contamination. The stock should be tested for mycoplasma contamination using at least one of the methods described in this document, then frozen for storage. For each test a new container from the stock should be thawed and used within 6 passages.

Indicator cell cultures should be grown on cover slips sub-

merged in culture dishes or equivalent containers for one day. Inoculate no less than 1 mL of the test sample (cell culture supernatant) into two or more of the culture dishes.

The test should include a negative (non-infected) control and two positive mycoplasma controls, such as *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains). Use an inoculum of 100 CFU or 100 CCU or less for the positive controls.

Incubate the cell cultures for 3 to 6 days at 36 ± 1ºC in an atmosphere of air containing 5 percent carbon dioxide.

Examine the cell cultures after fixation for the presence of mycoplasma by epifluorescence microscopy (400 to 600 times magnification or greater) using a DNA-binding fluorochrome, such as bisbenzimidazole or an equivalent stain. Compare the microscopical appearance of the test cultures with that of the negative and positive controls.

Procedure

1) Aseptically place a sterilized glass cover slip into each cell culture dish (35 mm diameter).

2) Prepare Vero cell suspension in Eagle's minimum essential medium containing 10 percent bovine calf serum at a concentration of 1×10^4 cells per 1 mL. The bovine calf serum should be tested and confirmed to be free from mycoplasma prior to use.

3) Inoculate aliquots of 2 mL of the Vero cell suspension into each culture dish. Ensure that the cover slips are completely submerged, and not floating on the surface of the culture medium. Incubate the cultures at 36 ± 1ºC in an atmosphere of air containing 5 percent carbon dioxide for one day, so that the cells are attached to the glass cover slip.

4) Replace 2 mL of the culture medium with fresh medium, then add 0.5 mL of the test sample (cell culture supernatant) to each of two or more culture dishes. Perform the same procedure for the positive (2 types of mycoplasma, such as *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) and *M. orale* (ATCC23714 or equivalent species or strains)) and negative controls.

5) Incubate the cultures for 3 to 6 days at 36 ± 1ºC in an atmosphere of air containing 5 percent carbon dioxide.

6) Remove the culture medium from the culture dishes, and add 2 mL of a mixture of acetic acid (100) and methanol (1:3) (fixative) to each dish; then, allow them to stand for 5 minutes.

7) Remove the fixative from each dish, then add the same amount of fixative again, and leave the dishes to stand for 10 minutes.

8) Remove the fixative and then completely air-dry all the dishes.

9) Add 2 mL of bisbenzamide fluorochrome staining solution to each culture dish. Cover the dishes and let them stand at room temperature for 30 minutes.

10) Aspirate the staining solution and rinse each dish with 2 mL of distilled water 3 times. Take out the glass cover slips and dry them.

11) Mount each cover slip with a drop of a mounting fluid. Blot off surplus mounting fluid from the edges of the cover slips.

12) Examine by epifluorescence microscopy at 400 to 600 times magnification or greater.

13) Compare the microscopic appearance of the test sample with that of the negative and positive controls.

14) The test result is judged to be positive if there are more than 5 cells per 1000 (0.5%) that have minute fluorescent spots that appear to surround, but are outside, the cell nucleus.

C. Polymerase Chain Reaction (PCR) Detection Method

The PCR method is a highly specific method that enables the detection of trace amounts of mycoplasma DNA, and has come to be widely used in recent years as a means of detecting mycoplasma contamination. However, the sensitivity and specificity depend on the procedure employed, and a positive result from PCR does not always indicate the presence of viable mycoplasma.

The PCR method is based on amplifying DNA extracted from the cell culture with specific primers so that the presence of the target DNA is detected. A two-step PCR (nested PCR) is recommended in order to increase sensitivity and specificity. The tests should include both a positive control (such as *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains) of 100 CFU or 100 CCU or less) and a negative control.

Mycoplasma DNA from the sample of cells or cell cultures is amplified using primers which should be able to amplify some common conserved mycoplasma DNA sequence. The amplification should be performed using an appropriate heat-resistant DNA polymerase, and suitable conditions. The amplified DNA can be identified after agarose gel electrophoresis, followed by ethidium bromide staining and UV irradiation of the gel.

For this method, it is important to use primers that are specific to mycoplasma by choosing base sequences that are well-conserved for a wide range of mycoplasma species, for example, the spacer region between the 16S-23S ribosome genes.

It is recommended that a two-step PCR using nested primers should be performed to increase the sensitivity and specificity, if the one-step PCR is negative.

The primers to be selected for the second stage of a two-step PCR are nested primers from the inner portion of the sequence. The outer and inner primers should have proven effectiveness and specificity as described in publications or be validated experimentally.

It is possible to increase the accuracy of the detection of mycoplasma DNA by performing PCR tests after cultivation of mycoplasma that may be present in samples using Vero cells.

The following is an example of a two-step PCR procedure. The reagents and reaction conditions in this example are not exclusive. If the suitability of other reagents and conditions is verified, they may be used. If another procedure is used, the procedure should be justified and documented in detail, and the information provided should include the sensitivity and specificity of the method.

Example Procedure

1. Preparation of template

1) Place 600 μL of the test cell suspension (if necessary, subcultured with Vero cells) in a tube and dissolve the cells with

0.1% SDS or an equivalent. Add an equal volume (600 μL) of TE (10 mmol/L tris-hydrochloric acid (pH 8.0), 1 mmol/L EDTA) buffer-saturated phenol, and mix.

2) Centrifuge at 15,000 min^{-1} for 5 minutes at room temperature.

3) Transfer 400 μL of the supernatant to another tube, and add 10 μL of 3 mol/L sodium acetate.

4) Add 1 mL (2.5 volumes) of ethanol (95) and stir thoroughly. Ice the mixture for 15 minutes, then centrifuge at 15,000 min^{-1} for 10 minutes at 4ºC.

5) Discard the supernatant and rinse the precipitate once or twice with 200 to 300 μL of 80% ethanol. Remove the rinse solution using a pipet. Centrifuge at 15,000 min^{-1} for 10 minutes at 4ºC, then remove the supernatant thoroughly and dry up the precipitate.

6) Dissolve the precipitate in 40 μL of distilled water.

2. Perform the same procedure for the positive and negative controls

3. First stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, outer primer, and reaction buffer solution (including Mg ions), and place 90 μL in each tube.

2) Add 10 μL of the template prepared as above to each tube containing the first stage PCR solution (90 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94ºC for 30 seconds, annealing at an appropriate temperature for the primer (55ºC for the primer in this example), and elongation at 72ºC for 2 minutes.

4. Second stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, inner primer, and reaction buffer solution (including Mg ions), and place 99 μL in each tube.

2) Add 1 μL of the first stage PCR product from each tube to a tube containing the second stage PCR solution (99 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94ºC for 30 seconds, annealing at an appropriate temperature for the primer (55ºC for the primer in this example), and elongation at 72ºC for 2 minutes.

5. Agarose gel electrophoresis

1) Mix 10 μL of each of the first stage and second stage PCR products with 2 μL of an appropriate dye as a migration marker, and perform 1% agarose gel electrophoresis.

2) Stain the gel with ethidium bromide and take a photograph under UV irradiation.

3) The test is judged to be positive if a DNA band is detected.

[An Example of Primer]

For mycoplasma detection

Outer primer

F1:5′-ACACCATGGGAG(C/T)TGGTAAT-3′

R1:5′-CTTC(A/T)TCGACTT(C/T)CAGACCCAAGG-CAT-3′

Inner primer

F2:5′-GTG(G/C)GG(A/C)TGGATCACCTCCT-3′

R2:5′-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3′

( ) indicates a mixture.

[PCR reaction solution]	[First stage]	[Second stage]
dNTP solution (1.25 mol each)	16 μL	16 μL
Primer (10 pmol/μL)	F1 2 μL	F2 2 μL
Primer (10 pmol/μL)	R1 2 μL	R2 2 μL
Heat-resistant DNA polymerase (1 U/μL)	2 μL	2 μL
Reaction buffer solution		
25 mmol/L magnesium chloride hexahydrate	8 μL	8 μL
10-fold buffer solution*	10 μL	10 μL
Sterile distilled water	50 μL	59 μL

*Composition of 10-fold buffer solution

2-amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.4)	100 mmol/L
Potassium chloride	500 mmol/L
Magnesium chloride hexahydrate	20 mmol/L
Gelatin	0.1 g/L

[Method of cultivating mycoplasma within Vero cells]

1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.

2) Into each cell culture dish (35 mm diameter), inoculate 2 mL of the Vero cell suspension (1×10^4 cells per 1 mL) in Eagle's minimum essential medium containing 10 percent bovine calf serum (tested in advance using the PCR method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at 36 ± 1ºC in an atmosphere of air containing 5 percent carbon dioxide for one day.

3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or 100 CCU or less of *M. hyorhinis* (ATCC29052, ATCC17981 or equivalent species or strains)) and negative controls.

4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 to 6 days at 36 ± 1ºC in an atmosphere of air containing 5 percent carbon dioxide.

Add the following:

32. Near Infrared Spectrometry

Near infrared spectrometry (NIR) is one of spectroscopic methods used to qualitatively and quantitatively evaluate substances from analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (wave numbers) between 750 and 2500 nm (13333 – 4000 cm^{-1}). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range (4000 to 400 cm^{-1}), primarily absorption of O-H, N-H, C-H and S-H that involve hydrogen atoms, in particular. For instance the asymmetrical stretching vibration of N-H occurs in the vicinity of 3400 cm^{-1}, but the absorption due to the first harmonic overtone occurs in the vicinity of 6600 cm^{-1} (wavelength 1515 nm), which is near double 3400 cm^{-1}.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid specimens, including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to specimens.

Conventional spectrometry, such as calibration curve method, is used as a method for analyzing near-infrared absorption spectrum whenever applicable. Ordinarily, however, chemometrics methods are used for analysis. Chemometrics ordinarily involve quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Chemometrics for near-infrared spectrometry includes various types of multivariate analysis such as multiple regression analysis, to perform qualitative or quantitative evaluation of active substances.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods for water determinations or substance verifications. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as a quality evaluation test method in routine tests.

Applications of near-infrared spectrometry in the pharmaceutical field include qualitative or quantitative evaluation of ingredients, additives or water contents of active substances or preparations. Furthermore, near-infrared spectrometry can also be used for evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online.

1. Equipment

Near-infrared spectrophotometers can either be a distributed near-infrared spectrophotometer or a Fourier transform near-infrared spectrophotometer[1)]. Interference filter-type near-infrared spectrophotometers that use interference filter in the spectrometry section are also available, however, this type of equipment is hardly used in the field of pharmaceutical quality control.

1.1 Distributed near-infrared spectrophotometer

This equipment is comprised of light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, display-record-output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner are used in the light source section. The sample section is comprised of a sample cell and a sample holder. Equipment that have an optical fiber section that is comprised of optical fibers and a collimator are equipped with a function for transmitting light to sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers.

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. Potential dispersive devices include prisms, diffraction grating, acousto-optical tunable filters (AOTF), or liquid crystal tunable filters (LCTF).

The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors (silicon, lead sulfide, indium-gallium-arsenic, indium-antimony), as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with single elements, but there are also occasions where array-type detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The data processing section performs data conversions and spectral analysis, etc. The display-record-output section outputs data, analysis results and data processing results to a printer.

1.2 Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally same as that of the distributed-type equipment described in Section 1.1, except for the spectrometry section and the signal processing section.

The spectrometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. Interferometers include Michelson interferometers, transept interferometers and polarization interferometers. The signal processing section is equipped with functions that are required for spectrometer, as well as a function for translating acquired interference waveform (interferogram) into absorption spectrum by Fourier transformation.

2. Determination

There are three types of measurement methods that are used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The selection of measurement methods relies on the shape of samples and applications. The transmittance method or diffuse reflectance method is used for solid samples, including fine particles. The transmittance method or transmittance reflectance method is used for liquid samples.

2.1 Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate T (%) or absorbance A with the transmittance method.

A sample is placed in the light path between a light source and a detector, the arrangement of which is ordinarily same as that of the spectroscopic method.

$T = 100\ t$

$T = I / I_0 = 10^{-\alpha cl}$

I_0: Incident light intensity

I: Transmitted light intensity

α: Absorptivity

c: Solution concentration

l: Layer length (sample thickness)

$A = -\log t = \log (1 / t) = \log (I_0 / I) = \alpha cl$

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1 – 5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids, including fine particles. It is also known as diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface conditions of samples.

2.2 Diffuse reflectance method

The ratio of the reflection light intensity I, emitted from the sample in a wide reflectance range and a control reflection light intensity I_r emitted from surface of a substance, is expressed as reflectance R (%) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples, including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance (A_r) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance ($1/r$) against wavelengths (wave numbers).

$R = 100\ r$

$r = I / I_r$

I: Reflection light intensity of light, diffuse reflected off the sample

I_r: Control reflection light intensity of light emitted from surface of reference substance

$A_r = \log (1 / r) = \log (I_r / I)$

The intensity of diffuse reflectance spectrum can also be expressed with the Kubelka-Munk (K-M) function. The K-M function is derived, based on the existence of a sample with sufficient thickness, and expressed in terms of light scattering coefficient, which is determined by absorptivity, grain size, shape and fill condition (compression).

This method is applied to solid samples, including fine particles, and requires a diffuse reflector.

2.3 Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect a light that has passed through a sample in order to take a measurement of transmittance reflectance rate, T^* (%). Light path must be twice the thickness of the sample. On the other hand, the light reflected off a mirror and enters into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with rough surface that causes diffuse reflectance is used instead of a mirror.

Transmittance reflectance absorbance (A^*) is obtained by the following formula with this method:

$T^* = 100\ t^*$

$t^* = I / I_T$

I: Intensity of transmitted and reflected light, in cases where a sample is placed

I_T: Intensity of reflected light, in cases where is no sample

$A^* = \log (1 / t^*)$

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 – 2 (transmittance of 79 – 1%), which provides the best linearity and S/N ratio of detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectrum

Following items must be considered as factors that can affect spectrum when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

3.1 Sample temperature

A significant change (wavelength shift, for example) can occur when the temperature varies by a several degree (°C). Care must be taken, particularly when the sample is a solution or contains water.

3.2 Water or residual solvent

Water or residual solvent contents of a sample, as well as water (humidity) in the environment wherein measurements are taken, can potentially significantly affect absorption band of the near-infrared range.

3.3 Sample thickness

The thickness of a sample is a factor for spectral changes and

therefore needs to be controlled at a certain thickness. A sample may be considered to be of adequate thickness for the diffuse reflectance method, however, if the thickness is less than a certain amount, for example, the sample may have to be placed on a support plate with high reflectance to take measurements by the transmittance reflectance method.

3.4 Fill condition of sample

The condition of sample fill can potentially affect spectrum, when taking measurements of samples that are solids or fine particles. Care must be taken when filling samples in a cell, to ensure that a certain amount is filled through a specific procedure.

3.5 Optical characteristics of samples

When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same sample, or a sample must be pulverized to ensure averaging of the sample. Grain size, fill condition, as well as roughness of surface can also affect fine particle samples.

3.6 Crystal forms

Variations in crystal structures (crystal forms) can also affect spectrum

In cases where multiple crystal forms exist, it is necessary to have consideration for characteristics of samples to be considered and care must be taken to ensure that even standard samples for calibration curve method have diversified distributions similar to that of samples that are subject to analysis.

3.7 Temporal changes in characteristics of samples

Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling, and such changes affect spectrum in a subtle manner. For instance even with identical samples, if elapsed times differ, then their characteristics of near-infrared spectrum can vary significantly. In creating calibration curves, therefore, measurements must be taken offline in a laboratory or online in manufacturing process (or inline) and samples for calibration curves must be prepared with adequate considerations for the passing of time before measurements are taken.

4 Control of equipment performance[2, 3)]

4.1 Accuracy of wavelengths (wave numbers)

The accuracy of wavelengths (wave numbers) of an equipment is derived from the deviation of substances for which peak absorption wavelengths (wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner:

1200 ± 1 nm (8300 ± 8 cm^{-1})

1600 ± 1 nm (6250 ± 4 cm^{-1})

2000 ± 1.5 nm (5000 ± 4 cm^{-1})

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (wave numbers) that are closest to the above 3 peaks are selected for suitability evaluations. A mixture of rare earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm, 1681 nm and 1971 nm.

Absorption peaks at 1155 nm, 1417 nm, 1649 nm, 2352 nm (layer length: 1.0 mm) can be used, when taking measurements with transmittance method that involve the use of dichloromethane as reference. The absorption peak of steam at 7306.7 cm^{-1} can be used with a Fourier transformation-type spectrophotometer, as its wave number resolution ability is high.

Other substances can also be used as reference, so long as their adequacy for the purpose can be verified.

4.2 Spectroscopic linearity

Appropriate standard plates, such as plate-shaped polymer impregnated with varying concentrations of carbon (carbon-doped polymer standards), can be used to evaluate spectroscopic linearity. In order to verify linearity, however, standard plates with no less than 4 levels of concentration within the reflectance of 10 – 90% must be used. When measurements are expected to be taken with absorbance of no less than 1.0, it is necessary to add standard plates with reflectance of either 2% or 5% or both.

In order to plot absorbance (A_{OBS}) of such standard plates at locations in the vicinity of wavelengths 1200 nm, 1600 nm and 2000 nm against absorbance (A_{REF}) assigned to each standard plate, verifications must be made to ensure that the gradient of linearity obtained are within the range 1.0±0.05 for each of these wavelengths and 0±0.05 for ordinate intercept.

4.3 Spectrophotometric noise

The spectrophotometric noise of the equipment can be checked using appropriate reflectance standard plates, such as white-colored reflecting ceramic tiles or reflective thermoplastic resin (such as polytetrafluoroethylene).

4.3.1 High flux noise

Spectrophotometric noise is evaluated by using standard plates with high reflectance, such as reflectance of 99%. Standard plates are used to take measurements for both samples and control samples. The average value obtained from calculation of mean square root (*RMS*) of noise for each 100 nm segments in the wavelength range of 1200 – 2200 nm must not be more than 0.3 x 10^{-3} and individual values must not exceed 0.8 x 10^{-3}.

$$RMS = \{1 / N \bullet \Sigma (A_i - A_m)^2\}^{1/2}$$

N: Number of measurement points per segment

A_i: Absorbance at each measurement point of segment

A_m: Average absorbance for segment

4.3.2 Low flux noise

Spectrophotometric noise is evaluated by using standard plates with low reflectance, such as reflectance of 10%, when the amount of light is low. In such cases, light source, optical system, detector and electronic circuit systems all have some impact on noise. Similar to the cases of high flux noise, the average value obtained from calculation of *RMS* for each 100 nm segments in the wavelength range of 1200 – 2200 nm must not be more than 1.0 x 10^{-3} and individual values must not exceed 2.0 x 10^{-3}.

5. Application to qualitative or quantitative analysis

Unlike in the infrared range, mainly harmonic overtones and combinations manifest as spectrum in the near-infrared range. Such absorbance spectrums are often observed as overlay of

absorption bands of functional groups and atomic groups. The near-infrared spectrometry, therefore, differs from conventional analysis methods and it is usually necessary to establish analysis methods that correspond to each application, by preparing model analysis methods using methodologies of chemometrics, such as multivariate analysis.

Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectrums, as well as overlay of absorption bands must be reduced by performing mathematical preprocesses, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics. While there are many chemometrics methodologies and mathematical preprocessing methods for data, appropriate combinations must be selected that suit the purposes of intended analysis.

Evaluation of validity based on analysis parameters is ordinarily required for the analysis validation when establishing a near-infrared analysis method. Selection of parameters that are appropriate for applications must be made for its intended use. Furthermore, following issues must be considered, in conformity with attributes of the near-infrared spectrometry.

(i) Whether or not wavelengths (wave numbers) intended for the particular analysis method, are suitable for evaluation of characteristics of a sample in performing analysis under given conditions.

(ii) Whether or not the method is adequately robust to deal with variables such as handling of samples (for instance fill condition for fine particle samples, etc.) and configuration matrix.

(iii) Whether or not about the same level of accuracy or precision can be obtained, in comparison with the existing and established analysis methods, which are available as standards.

(iv) Sustaining and managing performance of an analysis method, once established, are critical. Continuous and systematic maintenance and inspection work must therefore be implemented. Furthermore, it must be determined whether or not appropriate evaluation procedures are available to deal with change controls or implementation of re-validation on changes made in manufacturing processes or raw materials, as well as changes arising from replacement of major components in equipment.

(v) Whether or not there are appropriate evaluation procedures in place to verify validity of transferring implementation of an analysis, which presupposes the use of a specific equipment, from such originally intended equipment to another equipment (model transfer) for the purpose of sharing the analysis method.

5.1 Qualitative analysis

Qualitative analysis, such as verification of substances, is performed after preparing a reference library that includes inter-lot variations within tolerance range and chemometrics methodologies, such as multivariate analysis, have been established. Minute quality characteristic variations between lots can also be estimated by using this method.

Furthermore, multivariate analysis includes direct analysis methods that consider wavelengths (wave numbers) and absorptions as variables, such as wavelength correlation method, residual sum of squares, range sum of squares, along with factor analysis method, cluster analysis method, discriminant analysis method, as well as SIMCA (soft independent modeling of class analogy).

It is also possible to consider the overall near-infrared absorption spectrum as a single pattern and to identify parameters obtained by applying multivariate analysis methods or characteristic wavelength (wave number) peaks of the sample substance as indices for monitoring, for the purpose of manufacturing process control for active substances or preparations.

5.2 Quantitative analysis

Quantitative analysis uses spectrums of sample groups and analysis values obtained through the existing and established analysis methods, to obtain quantitative models with methodologies of chemometrics. These are used to calculate concentrations of individual ingredients and material values of samples being measured, using conversion formulas. Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method, main ingredient regression analysis method and PLS (partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample that are subject to analysis can be calculated, by plotting a calibration curve using the absorbance of a specific wavelength (wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).

Reference

1) General Rules for Near-infrared Spectrophotometric Analysis, JIS K 0134 (2002), Japanese Industrial Standards

2) Near-infrared Spectrophotometry, 2.2.40, European Pharmacopoeia 5.0 (2005)

3) Near-infrared Spectrophotometry, <1119>, US Pharmacopoeia 30 (2007)

Add the following:

33. Rapid counting of microbes using fluorescent staining

This chapter provides rapid methods using fluorescence staining for the quantitative estimation of viable microorganisms. Incubation on an agar medium has been widely used for quantitative estimation of viable microorganisms, but a number of environmental microorganisms of interest are not easy to grow in culture under usual conditions, thus new microbial detection methods based on fluorescence or luminescence have been developed. In the fluorescence staining method, microorganisms are stained with fluorescent dye, and can easily be detected and counted with various sorts of apparatus, such as a fluorescence microscope or flow cytometer. Methods are available to detect total microorganisms, including both dead and viable cells, or to detect only cells with a specified bioactivity

by choosing the dye reagent appropriately. Nucleic acid staining reagents, which bind with DNA or RNA, detect all cells containing nucleic acids, whether they are live or dead. This technique is the most fundamental for the fluorescence staining method. On the other hand, fluorescent vital staining methods target the respiratory activity of the microorganism and the activity of esterase, which is present universally in microorganisms. In the microcolony method, microcolonies in the early stage of colony formation are counted. The CFDADAPI double staining method and the microcolony method are described below. These methods can give higher counts than the other techniques, because these rapid and accurate techniques provide quantitative estimation of viable microorganisms based on a very specific definition of viability, which may be different from that implicit in other methods. The procedures of these methods described here may be changed as experience with the methods is accumulated. Therefore, other reagents, instruments and apparatus than those described here may also be used if there is a valid reason for so doing.

1. CFDADAPI double staining method

Fluorescein diacetate (FDA) reagents are generally used for the detection of microorganisms possessing esterase activity. These reagents are hydrolyzed by intracellular esterase, and the hydrolyzed dye exhibits green fluorescence under blue excitation light (about 490 nm). Modified FDAs such as carboxyfluorescein diacetate (CFDA) are used because of the low stainability of gramnegative bacteria with FDA. The principle of the CFDADAPI double staining method, which also employs a nucleic acid staining reagent, 4′,6-diamidino-2-phenylindole (DAPI), is as follows.

The nonpolar CFDA penetrates into the cells and is hydrolyzed to fluorescent carboxyfluorescein by intracellular esterase. The carboxyfluorescein is accumulated in the living cells due to its polarity, and therefore green fluorescence due to carboxyfluorescein occurs when cells possessing esterase activity are illuminated with blue excitation light. No fluorescent carboxyfluorescein is produced with dead cells, since they are unable to hydrolyze CFDA. On the other hand, DAPI binds preferentially to the adenine and thymine of DNA after penetration into both viable and dead microorganisms, and consequently all of the organisms containing DNA exhibit blue fluorescence under ultraviolet excitation light. Therefore, this double staining method enables to count specifically only live microorganisms possessing esterase activity under blue excitation light, and also to determine the total microbial count (viable and dead microorganisms) under ultraviolet excitation light.

1.1 Apparatus

1.1.1 Fluorescence microscope or fluorescence observation apparatus . Various types of apparatus for counting fluorescencestained microorganisms are available. Appropriate filters are provided, depending on the fluorescent dye reagents used. A fluorescence microscope, laser microscope, flow cytometer, and various other types of apparatus may be used for fluorescence observation.

1.2 Instruments

(i) Filtering equipment (funnels, suction flasks, suction pumps)

(ii) Membrane filters (poresize: 0.2 μm); A suitable filter that can trap particles on the surface can be used such as polycarbonate filter, alumina filter, etc.

(iii) Glass slide

(iv) Cover glass

(v) Ocular micrometer for counting (with 10 x 10 grids)

1.3 Procedure

An example of the procedure using fluorescence microscope is described below.

1.3.1 Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

1.3.2 Filtration

Set a membrane filter (poresize: 0.2 μm) on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

1.3.3 Staining

Pour sufficient amount of buffer solution for CFDA staining, mixed to provide final concentration of 150 μg/mL of CFDA and 1 μg/mL of DAPI, into the funnel of the filtering equipment and allow staining in room temperature for 3 minutes, then filter the liquid by suction. Pour in sufficient amount of aseptic water, suction filter and remove excess fluorescent reagent left on the filter. Thoroughly dry the filter.

1.3.4 Slide preparation

Put one drop of immersion oil for fluorescence microscope on the glass slide. Place the air dried filter over it, with the filtering side on the top. Then put one drop of immersion oil for fluorescence microscope on the surface of the filter, place a cover glass. Put another drop of immersion oil for fluorescence microscope on the cover glass when using an oilimmersion objective lens.

1.3.5 Counting

Observe and count under fluorescence microscope, with 1000 magnification. In case of CFDADAPI double staining method, count the microorganisms (with esterase activity) exhibiting green fluorescence under the blue excitation light first to avoid color fading by the ultraviolet light, then count the microorganisms (with DNA) exhibiting blue fluorescence under the ultraviolet excitation light in the same microscopic field. Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among 100 grids observed through an ocular micrometer of the microscope, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers. The amount of the sample to be filtered must be adjusted so that the cell number per field is between 10 and 100. It might be necessary to reprepare the sample in certain instances. (In such case that the average count number is not more than 2 organisms per field, or where more than 5 fields are found which have no organism per field, it is assumed that the microorganism count is below the detection limit.).

Number of microbes (cells/mL)
= {(average number of microbes per visual field) x (area of filtration)} / {(amount (mL) of sample filtered) x (area of one microscopic field)}

1.4 Reagents and test solutions

(i) Aseptic water

Filter water through a membrane filter with 0.2 μm pore size to remove particles, then sterilize it by heating in an autoclave at 121ºC for 15 minutes. Water for injection may be used.

(ii) CFDA solution, 10 mg/mL

Dissolve 50 mg of CFDA in dimethylsulfoxide to prepare a 5 mL solution. Store at -20°C in light shielded condition.

(iii) Buffer solution for CFDA staining

Mix 5 g of sodium chloride with 0.5 mL of 0.1 mol/L ethylenediaminetetraacetic aciddisodium dihydrogen test solution and diluted disodium hydrogen phosphate test solution (1 in 3) to prepare 100 mL of solution. Add sodium dihydrogen phosphate dihydrate solution (1 in 64) to adjust the pH level to 8.5. Filter the solution through a membrane filter with a pore size of 0.2 μm.

(iv) DAPI solution, 10 μg/mL

Dissolve 10 gm of DAPI in 100 mL of aseptic water. Dilute this solution 10 times with aseptic water and filter through a membrane filter with a pore size of 0.2 μm. Store at 4°C in light shielded condition.

(v) Immersion oil for fluorescence microscope

2. Microcolony method

Microcolonies, which are in early stages of colony formation, are fluorescently stained, then observed and counted under fluorescence microscope or other suitable systems. This method enables to count the number of proliferative microorganisms, with short incubation time. In this method, the organisms are trapped on a membrane filter, the filter is incubated on a medium for a short time, and the microcolonies are counted. By this method, even colonies which are undetectable with the naked eye can be identified, so viable organisms can be counted rapidly and with high precision.

Various nucleic acid staining reagents can be used for staining of microcolonies.

2.1 Apparatus

2.1.1 Fluorescence microscope or fluorescence observation apparatus.

Various types of apparatus for counting fluorescencestained microorganisms are available. Appropriate filters are provided, depending on the fluorescence dye reagents used. A fluorescence microscope, laser microscope and various other types of apparatus may be used for fluorescence observation.

2.2 Instruments

(i) Filtering equipment (funnels, suction flasks, suction pumps)

(ii) Membrane filters (pore size: 0.2 μm); A suitable filter that can trap particles on the surface can be used such as polycarbonate filter, alumina filter, etc.

(iii) Glass slide

(iv) Cover glass

(v) Filter paper (No. 2)

(vi) Ocular micrometer for counting (with 10 x 10 grids)

2.3 Procedure

An example of the procedure using a fluorescence microscope is described below.

2.3.1 Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

2.3.2 Filtration

Set a membrane filter (pore size: 0.2 μm)on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

2.3.3 Incubation

Remove the filter from the filtering equipment and place it with filtering side facing up on a culture medium avoiding formation of airbubbles between the filter and the medium. Incubate at a suitable temperature for appropriate hours in a dark place. It should be noted that the appropriate incubation conditions (such as medium, incubation temperature and/or incubation time) are different, depending on the sample.

2.3.4 Fixation

Soak filter paper with an appropriate amount of neutral buffered formaldehyde test solution, then place the filter that has been removed from the culture medium on top with filtering side up, and allow to remain at room temperature for more than 30 minutes to fix the microcolonies.

2.3.5 Staining

Soak filter paper with an appropriate amount of staining solution (such as1 μg/mL of DAPI, 2% polyoxyethylenesorbitan monolaurate), then place the filter on top with filtering side up, and then leave at room temperature, light shielded for 10 minutes to stain microcolonies. Wash the filter by placing it with the filtering side facing up on top of a filter paper soaked with aseptic water for 1 minute. Thoroughly air dry the filter.

2.3.6 Slide preparation

Put one drop of immersion oil for fluorescence microscope on the slide glass. Place an air dried filter over it, with the filtering side on the top. Then, put one drop of immersion oil for fluorescence microscope on top, place a cover glass.

2.3.7 Counting

Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among the 100 grids observed through an ocular micrometer of the microscope, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers.

(In such case that the average count number is not more than 2 microcolonies per field, or where more than 5 fields are found which have no microcolony per field, it is assumed that the microorganism count is below the detection limit.).

Number of microcolonies (cells/mL)
= {(average number of microcolonies per visual field) x (area of filtration)} / {(amount (mL) of sample filtered) x (area of one microscopic field)}

2.4 Reagents and test solutions

(i) Aseptic water

Filter water through a membrane filter with 0.2 μm pore size to remove particles, and sterilize it by heating in an autoclave at 121ºC for 15 minutes. Water for injection may be used.

(ii) Staining solution

Dissolve 10 gm of DAPI in 100 mL of aseptic water. Dilute the solution 10 times with aseptic water and filter through a membrane filter with pore size of 0.2 μm. Store at 4°C in light

shielded condition. Dissolve polyoxyethylene sorbitan monolaurate to the final concentration of 2%, when using.
(iii) Neutral buffered formaldehyde solution (4w/v% formaldehyde solution; neutrally buffered).
(iv) Immersion oil for fluorescence microscope

34. System Suitability

In order to ensure the reliability on the results of drug analyses, it is essential to verify that the test method to be applied to the test, including the method prescribed in the Japanese Pharmacopoeia (JP), can give the results adequate for its intended use using the analytical system in the laboratory in which the test is to be performed, then to carry out system suitability testing for confirming that the analytical system maintains the state suitable for the quality test.

1. Definition and role of system suitability

"System Suitability" is the concept for ensuring that the performance of the analytical system is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system.

Usually, system suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test methods of drugs. The results of drug analyses are not acceptable unless the requirements of system suitability have been met.

System suitability testing is an integral part of test methods using analytical instruments, and based on the concept that the equipments, electronic data processing systems, analytical operations, samples to be analyzed and operators constitute an integral system that can be evaluated, when the test procedures and acceptance criteria of system suitability testing are prescribed in the test methods.

2. Points to consider in setting system suitability

Parameters of system suitability testing to be prescribed in the test method depend on the intended use and type of analytical method. Since system suitability testing is to be carried out in a routine manner, it is preferable to select the parameters necessary for ensuring that the analytical system maintains the state suitable for the analysis of the drug and to prescribe its test procedure able to carry out easily and rapidly.

For example, in the case of quantitative purity tests using liquid chromatography or gas chromatography, the evaluation of parameters such as "System performance" (to confirm the ability to analyze target substance specifically), "System repeatability" (to confirm that the degree of variation in the analytical results of target substance in replicate injections is within the allowable limit) and "Test for required detectability" (to confirm the linearity of chromatographic response around the specification limit) are usually required.

The followings are supplements to the section of system suitability prescribed in "Liquid Chromatography".

(1) Allowable limit of system repeatability

It is described in the section of system suitability in "Liquid Chromatography" that "In principle, total number of replicate injections should be 6.", and "The allowable limit of "System repeatability" should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test."

Based on the above description, an allowable limit of system repeatability for 6 replicate injections should be set in consideration with the following descriptions. However, in the case that the test method prescribed in the JP monograph is used for the test, the allowable limit of system repeatability prescribed in the monograph should be applied.

Assay for drug substance (for drug substance with the content nearby 100%): An adequate allowable limit should be set at the level that the chromatographic system is able to give the precision suitable for the evaluation of variation in the content of active ingredient within and among the batches of drug substance. For example, the allowable limit of "not more than 1.0%" is usually recommended for the drug substances whose width of content specification are not more than 5%, as is in the case of content specification of 98.0-102.0% which is often observed in the assay using liquid chromatography.

Assay for drug products: An adequate allowable limit should be set considering the width of content specification of the drug product and the allowable limit prescribed in the assay of drug substance (when the drug product is analyzed by a method with the same chromatographic conditions as those used for the analysis of drug substance).

Purity test for related substances: An adequate allowable limit should be set considering the concentration of active ingredients in the solution used for the system suitability testing. In the case that a solution with active ingredient concentration of 0.5-1.0% is used for the test of system repeatability, an allowable limit of "not more than 2.0%" is usually recommended.

Recommendations for allowable limits described above should not be applicable to gas chromatography.

(2) Method for decreasing the number of replicate injections without losing the quality of system repeatability testing

It is described in the section of system suitability in "Liquid Chromatography" that "In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of "System repeatability" which can guarantee a level of "System repeatability" equivalent to that at 6 replicate injections."

In consideration of the above description, a method for decreasing the number of replicate injections without losing the

quality of system repeatability testing is adopted. One can set the test for system repeatability with reduced number of replicate injections by utilizing this method, if necessary, and can also apply it as an alternative for the method prescribed in a monograph.

The following table shows the allowable limits to be attained in the test at 3-5 replicate injections (n=3-5) to keep the quality of test equivalent to that of test at n=6.

However, it should be kept in mind that since decrease in the number of replicate injections results in increase in the weight of each injection, it becomes more important to perform the test by the experienced operator, and to maintain the equipment in a suitable state.

Table Allowable limits to be attained in the test at 3-5 replicate injections (n=3-5) to keep the quality of test equivalent to that of test at n=6 *

		Allowable limit (RSD)					
Allowable limit prescribed in the test of n=6		1%	2%	3%	4%	5%	10%
Allowable limit to be attained	n=5	0.88%	1.76%	2.64%	3.52%	4.40%	8.81%
	n=4	0.72%	1.43%	2.15%	2.86%	3.58%	7.16%
	n=3	0.47%	0.95%	1.42%	1.89%	2.37%	4.73%

* The probability for inadequate analytical systems to meet the requirements of system suitability testing, is supposed to be 5%.

3. Points to consider at the change of analytical system (Change control of analytical system)

When the test method and analytical system verified is continuously used for the quality test without any change, it is sufficient to confirm the compliance to the requirements of system suitability at every series of drug analysis.

However, when the test is performed for a long period, a situation in which some changes in the analytical system are inevitable, may occur. These changes don't affect the quality of the product itself, but they affect the scale in the evaluation of product quality. If the change in the analytical system may induce a significant deviation of the scale, it may lead to the acceptance of products with inadequate quality and/or the rejection of products with adequate quality. Thus, at the time of change in the analytical system, it is necessary to check whether the change is appropriate or not, to avoid the deviation of the scale in the evaluation of product quality.

In the case of the change of test method, it is required to perform an adequate validation depending on the extent of the change.

On the other hand, in the case of the change of analytical system in a laboratory, such as renewal of apparatus or column of liquid chromatography, and the change of operator, it is necessary to perform at least system suitability testing using the system after change, and to confirm that the equivalency of the results before and after change.

In the case that equivalent results would not be obtained after change, for example, when a renewal of column of liquid chromatograph may induce a significant change of elution pattern, such as the reversal of elution order between target ingredient of the test and substance for checking resolution, it is required to perform a revalidation of the analytical system for the test using new column, since it is uncertain whether the specificity and/or other validation characteristics necessary for estimating target ingredient is kept or not.

35. Powder Fineness

This classification is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

A simple descriptive classification of powder fineness is provided in this chapter. Sieving is most suitable where a majority of the particles are larger than about 75 μm, although it can be used for some powders having smaller particle sizes where the meth——od can be validated. Light diffraction is also a widely used technique for measuring the size of a wide range of particles.

Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterized in the following manner:

x_{90}: particle size corresponding to 90% of the cumulative undersize distribution

x_{50}: median particle size (ie: 50% of the particles are smaller and 50% of the particles are larger)

x_{10}: particle size corresponding to 10% of the cumulative undersize distribution

It is recognized that the symbol d is also widely used to designate these values. Therefore, the symbols d_{90}, d_{50}, d_{10} may be used.

The following parameters may be defined based on the cumulative distribution.

$Q_r(x)$: cumulative distribution of particles with a dimension less than or equal to x where the subscript r reflects the distribution type

r	Distribution Type
0	Number
1	Length
2	Area
3	Volume

Therefore, by definition:

$Q_r(x) = 0.90$ when $x = x_{90}$

$Q_r(x) = 0.50$ when $x = x_{50}$

$Q_r(x) = 0.10$ when $x = x_{10}$

An alternative but less informative method of classifying powder fineness is by use of the descriptive terms in the following table.

Classification of Powders by Fineness		
Descriptive Term	x_{50} (μm)	Cumulative Distribution by volume basis, $Q_3(x)$
Coarse	>355	$Q_3(355) < 0.50$
Moderately Fine	180 – 355	$Q_3(180) < 0.50$ and $Q_3(355) \geq 0.50$
Fine	125 – 180	$Q_3(125) < 0.50$ and $Q_3(180) \geq 0.50$
Very Fine	≤125	$Q_3(125) \geq 0.50$

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