The Ministry of Health, Labour and Welfare Ministerial Notification No. 316

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, 2007. 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, 2007 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, 2009. 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, 2007 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, 2009. Further, Standards listed in the section 9.01 Reference Standards of the General Tests, Processes and Apparatus of the previous Pharmacopoeia at the end of application of this notification may be treated under the previous regulation irrespective of the prescription of the section 9.01(1) Reference Standards of the General Tests, Processes and Apparatus of the new Pharmacopoeia.

Yoichi Masuzoe
The Minister of Health, Labour and Welfare

September 28, 2007

(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 I to the Japanese Pharmacopoeia Fifteenth Edition from General Notice to Ultraviolet-visible Reference Spectra (pp. 1789 – 1997).

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, three working groups under the Panel on Medicinal Chemicals are established to expedite discussion of revision drafts of Monographs.

In the Committee on JP, Takao Hayakawa took the role of chairman from March 2006 to September 2007.

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 Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between September 2005 and March 2007, were prepared for a supplement to the JP 15. They were examined by the Committee on JP in April 2007, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2007, 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 (7); Sub-committee on the Principles of Revisions (6); Panel on Medicinal Chemicals (33, including the working groups); Panel on Antibiotics (9); Panel on Biologicals (8); Panel on Crude Drugs (17); Panel on Pharmaceutical Excipients (7); Panel on Physico-Chemical Methods (12); Panel on Prepara-

tions (10); Panel on Physical Methods (8); Panel on Biological Tests (7); Panel on Nomenclature (9); Panel on International Harmonization (2); and Panel on Pharmaceutical Water (7).

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 Japan Kampo Medicine Manufacturers' Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Pharmaceutical Manufacturers Association, and the Japan Oilseeds Processors Association.

In consequence of this revision, the JP 15th Edition carries 1567 articles, owing to the addition of 90 articles and the deletion of 6 articles.

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

1. The Supplement I to JP 15th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; 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 Supplement I.

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 abbreviations used for the principal units, "mol", "mmol", "mmol/L" and "Pa·s" were added to and "pH" was deleted from the paragraph 9 of the General Notices.

8. The following items of the General Rules for Preparations were revised:
(1) Extracts
(2) Ophthalmic Ointments
(3) Tinctures
(4) Ophthalmic Solutions
(5) Fluidextracts

9. The following item was added to the General Tests, Processes and Apparatus:
6.11 Foreign Insoluble Matter Test for Ophthalmic Solutions

10. The following items of the General Tests, Processes and Apparatus were revised:
(1) 1.09 Qualitative Tests
(2) 2.01 Liquid Chromatography
(3) 2.02 Gas Chromatography
(4) 2.48 Water Determination (Karl Fisher Method)
(5) 2.49 Optical Rotation Determination
(6) 4.01 Bacterial Endotoxins Test
(7) 4.05 Microbial Limit Test
(8) 6.01 Test for Metal Particles in Ophthalmic Ointments
(9) 6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions
(10) 6.10 Dissolution Test

11. The following Reference Standards were newly added:
Amlexanox
Amlodipine Besilate
Clobetasol Propionate
Enalapril Maleate
Manidipine Hydrochloride
Mizoribine
Nabumetone
Nizatidine
Ozagrel Sodium
Vincristine Sulfate
Zidovudine

12. The following Reference Standards were deleted.
Fosfestrol
Hypromellose Phthalate
Sulfinpyrazone
Tubocurarine Chloride Hydrochloride

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 preparations having a corresponding monograph of their principal material substances.

17. The following monographs were added:
Acemetacin
Alminoprofen

Alminoprofen Tablets
Alprostadil Injection
Amikacin Sulfate Injection
Amlexanox
Amlexanox Tablets
Amlodipine Besilate
Amosulalol Hydrochloride
Amosulalol Hydrochloride Tablets
Ampicillin Sodium for Injection
Azelastine Hydrochloride
Aztreonam for Injection
Benzylpenicillin Potassium for Injection
Biotin
Bisoprolol Fumarate
Bisoprolol Fumarate Tablets
Bucillamine Tablets
Buformin Hydrochloride
Buformin Hydrochloride Enteric-coated Tablets
Buformin Hydrochloride Tablets
Buprenorphine Hydrochloride
Cefadroxil Capsules
Cefadroxil for Syrup
Cefazolin Sodium for Injection
Cefmetazole Sodium for Injection
Ceftazidime for Injection
Cetirizine Hydrochloride
Cetirizine Hydrochloride Tablets
Chlorphenesin Carbamate Tablets
Cibenzoline Succinate
Cibenzoline Succinate Tablets
Cilazapril Hydrate
Cilazapril Tablets
Clindamycin Phosphate Injection
Clobetasol Propionate
Clorazepate Dipotassium
Clorazepate Dipotassium Capsules
L-Cysteine
L-Cysteine Hydrochloride Hydrate
Domperidone
Doxorubicin Hydrochloride for Injection
Emorfazone
Enalapril Maleate
Enalapril Maleate Tablets
Erythromycin Enteric-Coated Tablets
Etizolam Fine Granules
Etizolam Tablets
Felbinac
L-Glutamine
Griseofulvin Tablets
Ibudilast
Isoxsuprine Hydrochloride
Isoxsuprine Hydrochloride Tablets
Itraconazole
Josamycin Tablets
Labetalol Hydrochloride
Labetalol Hydrochloride Tablets
Manidipine Hydrochloride
Manidipine Hydrochloride Tablets
Minocycline Hydrochloride for Injection
Mitomycin C for Injection
Mizoribine
Mizoribine Tablets
Nabumetone
Nabumetone Tablets
Nafamostat Mesilate
Nizatidine
Nizatidine Capsules
Omeprazole
Ozagrel Sodium
Ozagrel Sodium for Injection
Peplomycin Sulfate for Injection
Piperacillin Hydrate
Rokitamycin Tablets
L-Serine
Sodium Starch Glycolate
Tobramycin Injection
L-Tyrosine
Ubenimex
Zidovudine
Aralia Rhizome
Powdered Corydalis Tuber
Crataegus Fruit
Hangekobokuto Extract
Keishibukuryogan Extract
Leonurus Herb
Lilium Bulb
Peucedanum Root
Powdered Turmeric

18. The following monographs were revised:
Acetylcholine Chloride for Injection
Ajimaline Tablets
Aminophylline Injection
Amitriptyline Hydrochloride Tablets
L-Arginine Hydrochloride Injection
Ascorbic Acid Injection
Baclofen Tablets
Betahistine Mesilate
Bisacodyl Suppositories
Calcium Chloride Injection
Calcium Folinate
Camostat Mesilate
Cefalotin Sodium
Cefatrizine Propylene Glycolate
Chlordiazepoxide Tablets
Chlorphenesin Carbamate
Chlorpromazine Hydrochloride Injection

Chlorpromazine Hydrochloride Tablets
Chlorpropamide Tablets
Cilostazol Tablets
Creosote
Cyanocobalamin
Cyanocobalamin Injection
Deferoxamine Mesilate
Dehydrocholic Acid Injection
Deslanoside Injection
Dextran 40
Anhydrous Dibasic Calcium Phosphate
Dibasic Calcium Phosphate Hydrate
Dopamine Hydrochloride Injection
Edrophonium Chloride Injection
Ephedrine Hydrochloride Injection
Ephedrine Hydrochloride Tablets
Famotidine for Injection
Faropenem Sodium Hydrate
Faropenem Sodium Tablets
Faropenem Sodium for Syrup
Folic Acid Injection
Folic Acid Tablets
Fructose Injection
Gabexate Mesilate
Glucose Injection
Hydralazine Hydrochloride Tablets
Hypromellose Phthalate
Idoxuridine Ophthalmic Solution
Imipramine Hydrochloride Tablets
Indometacin Capsules
Isotonic Sodium Chloride Solution
Anhydrous Lactose
Levallorphan Tartrate Injection
Magnesium Sulfate Injection
D-Mannitol Injection
Medazepam
Mefruside Tablets
Methyldopa Tablets
Morphine Hydrochloride Tablets
Neostigmine Methylsulfate Injection
Nicardipine Hydrochloride Injection
Nicorandil
Nicotinic Acid Injection
Noradrenaline Injection
Papaverine Hydrochloride Injection
Pethidine Hydrochloride Injection
Prednisolone Sodium Succinate for Injection
Protamine Sulfate
Protamine Sulfate Injection
Pyridoxine Hydrochloride Injection
Reserpine Injection
Riboflavin Sodium Phosphate Injection
Ringer's Solution
Roxithromycin
Salicylic Acid
Sodium Bicarbonate Injection
10% Sodium Chloride Injection
Sodium Citrate Injection for Transfusion
Sodium Thiosulfate Injection
Sulbactam Sodium
Sulpyrine Injection
Sultamicillin Tosilate Hydrate
Suxamethonium Chloride for Injection
Suxamethonium Chloride Injection
Talc
Teceleukin for Injection (Genetical Recombination)
Thiamine Chloride Hydrochloride Injection
Thiopental Sodium for Injection
Tipepidine Hibenzate Tablets
Trimetazidine Hydrochloride
Vincristine Sulfate
Water for Injection
Xylitol Injection
Alpinia Offcinarum Rhizome
Anemarrhena Rhizome
Angelica Dahurica Root
Apricot Kernel
Asiasarum Root
Asparagus Tuber
Atractylodes Rhizome
Powdered Atractylodes Rhizome
Belladonna Extract
Calumba
Powdered Calumba
Cimicifuga Rhizome
Clematis Root
Cnidium Rhizome
Powdered Cnidium Rhizome
Condurango Fluidextract
Coptis Rhizome
Powdered Coptis Rhizome
Corydalis Tuber
Cyperus Rhizome
Powdered Cyperus Rhizome
Dioscorea Rhizome
Powdered Dioscorea Rhizome
Fritillaria Bulb
Gastrodia Tuber
Gentian
Powdered Gentian
Glehnia Root
Glycyrrhiza Extract
Crude Glycyrrhiza Extract
Hochuekkito Extract
Imperata Rhizome
Ipecac

Powdered Ipecac
Japanese Gentian
Powdered Japanese Gentian
Japanese Valerian
Powdered Japanese Valerian
Kakkonto Extract
Kamishoyosan Extract
Lindera Root
Lithospermum Root
Lycium Bark
Magnolia Bark
Powdered Magnolia Bark
Mulberry Bark
Notopterygium Rhizome
Nuphar Rhizome
Nux Vomica Extract
Panax Japonicus Rhizome
Powdered Panax Japonicus Rhizome
Peach Kernel
Powdered Peach Kernel
Perilla Herb
Platycodon Fluidextract
Polygala Root
Powdered Polygala Root
Polygonum Root
Polyporus Sclerotium
Powdered Polyporus Sclerotium
Processed Aconite Root
Powdered Processed Aconite Root
Processed Ginger
Rehmannia Root
Ryokeijutsukanto Extract
Saposhnikovia Root
Saussurea Root
Scopolia Extract
Scopolia Rhizome
Scutellaria Root
Powdered Scutellaria Root
Senega
Powdered Senega
Smilax Rhizome
Powdered Smilax Rhizome
Sophora Root
Powdered Sophora Root
Turmeric
Uva Ursi Fluidextract
Zedoary

19. The following monographs were deleted:
Fosfestrol
Fosfestrol Tablets
Sulfinpyrazone
Sulfinpyrazone Tablets
Tubocurarine Chloride Hydrochloride Hydrate
Tubocurarine Chloride Hydrochloride Injection

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

Norio Aimi
Fumiaki Akahori
Mitsuo Aoki
Kiichi Aonuki
Nobuo Aoyagi**
Keiko Arimoto
Hiroshi Asama
Kazuhide Ashizawa
Shinichiro Aso
Yukio Aso
Takashi Bamba
Makoto Emura
Hiroyuki Fuchino
Shigeyuki Fujikura
Akihiko Fujise
Goro Funamoto
Yukihiro Goda
Ruri Hanajiri
Kouji Hasegawa
Mitsuru Hashida
Rika Hatano
Takao Hayakawa*
Masahiro Hayashi
Yoshinori Hayashi
Kenji Higuchi
Fusayoshi Hirayama
Yukio Hiyama
Kunimoto Hotta
Masashi Hyuga
Nobukazu Igoshi
Kenichi Inui
Akiko Ishii
Yuji Ito
Takashi Itoh
Masao Izaki
Kenichi Izutsu
Akemi Kai
Kazuaki Kakehi
Takemine Kanai
Motoko Kanke
Hirohito Katayama
Noriko Katori
Nobuo Kawahara
Toru Kawanishi
Yoshihiko Kawarasaki
Nana Kawasaki
Toshisuke Kawasaki
Yoshiaki Kawashima
Keiji Kijima
Yuuichi Kikuchi
Mitsukazu Kitada
Fumiyuki Kiuchi
Junko Kizu
Takashi Kobayashi
Masayoshi Kohase
Shigeo Kojima
Hiroyasu Kokubo
Katsuko Komatsu
Akio Komura
Tetsuya Konda
Toshifumi Konda
Kenji Kondo
Seizo Kondo
Takao Kunisada
Masaaki Kurihara
Fumiyo Kusu
Kumiko Kusuyama
Masako Maeda
Midori Makita
Yoshihisa Matsuda
Norio Matsuki
Eiichi Mikami
Tsuyoshi Miyagawa
Satoshi Minobe
Tsuyoshi Miura
Hiroto Miyamoto
Naoki Miyata
Michinao Mizugaki
Taiichi Mizuta
Kaoru Morikawa
Osamu Morita
Takashi Morita
Toshimi Murai
Masashi Muroi
Hiroaki Nagashige
Shinsaku Nakagawa
Emi Nakajima
Hiroshi Nakamura
Tatsuya Nakano
Tatsumi Nakashima
Mitsuo Nanaura
Masaaki Naotsuka
Masao Nasu
Shingo Niimi
Hiroshi Nishimura
Shukichi Ochiai
Atsuyuki Ohtsuki
Ryozo Oishi

Minoru Okada
Satoshi Okada
Kimiya Okazaki
Tsuneo Okubo
Haruhiro Okuda
Masami Otsuka
Tadashi Ouchi
Kazuhiro Owada
Kenji Saiki
Yoshikazu Sakagami
Eiji Sakai
Tomoaki Sakamoto
Hideki Sasaki
Hiroshi Sasaki
Tsuguo Sasaki
Motoyoshi Satake
Kyoko Sato
Michiko Sekiguchi
Setsuko Sekita
Yasuo Shimada
Kesamitsu Shimizu
Kyoko Shimura
Osamu Shirota
Hisashi Sonobe
Shoko Sueyoshi
Shinji Sugaya
Hisakazu Sunada
Hideyo Suzuki
Yoshikazu Takahashi
Toshio Takachi
Kikuo Takatera
Tadahiro Takeda
Yasushi Takeda
Toyoshige Tanabe
Haruo Tanaka
Toshihiro Tanaka
Kenichi Tanamoto**
Tsuyoshi Tanimoto
Susumu Terabayashi
Reiko Teraoka
Keijiro Terashita
Masafumi Teshigawara
Hiroshi Tokunaga
Kiyoshi Tomioka
Motowo Tomita
Hideya Tsuge
Yosuke Tsuji
Eriko Uchida
Yoshimasa Uehara
Kazuichi Umemoto
Haruo Watanabe
Takehiko Yajima
Teruhide Yamaguchi
Keiichi Yamamoto
Keiji Yamamoto
Tosuke Yamamoto
Takeshi Yamazaki
Masato Yasuhara
Hikaru Yoden
Chikako Yomota
Hitoo Yoshida
Sumie Yoshioka

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

GENERAL NOTICES

Change the paragraph 9 to read:

9. The following abbreviations are used for the principal units.

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

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

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, 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, 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, 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 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, Red Ginseng, Rehmannia Root, Rhubarb, Rice Starch, Rose Fruit, Rosin, 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 RULES FOR PREPARATIONS

7. Extracts

Change (1) to read:

(1) Extracts are prepared by evaporating the extractives of crude drugs. Generally, there are two kinds of Extracts which are:

(i) viscous extracts (ii) dry extracts

8. Fluidextracts

Change (4) to read:

(4) Unless otherwise specified, Fluidextracts meet the requirements of the Heavy Metals Limit Test *<1.07>* when the test solution and the control solution are prepared as follows.

Test solution: Ignite 1.0 g of Fluidextracts to ash, warm with 3 mL of dilute hydrochloric acid, filter, and wash the residue with two 5 mL portions of water. Add 1 drop of phenolphthalein TS to the combined filtrate and washings, add ammonia TS dropwise until the solution becomes a pale red, filter, if necessary, and add 2 mL of dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid as directed in the preparation of test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

17. Ophthalmic Ointments

Change (5) to read:

(5) Unless otherwise specified, Ophthalmic Ointments meet the requirements of the Test for Metal Particles in Ophthalmic Ointments *<6.01>*.

18. Ophthalmic Solutions

Change (8), (9) to read:

(8) Ophthalmic Solutions prepared as aqueous solution and aqueous vehicles attached to Ophthalmic Solutions, unless otherwise specified, meet the requirement of the Foreign Insoluble Matter Test for Ophthalmic Solutions *<6.11>*. The containers of Ophthalmic Solutions should have a transparency which does not interfere with the test for foreign matter.

(9) Unless otherwise specified, Ophthalmic Solutions meet the Insoluble Particulate Matter Test for Ophthalmic Solutions *<6.08>*.

27. Tinctures

Change (2) to read:

(2) Unless otherwise specified, Tinctures are usually prepared from coarse powder or fine cuttings of crude drug substance(s) either by maceration or by percolation as described below.

Maceration: Place crude drugs in a suitable container, and add the total volume or about three-fourths of the total volume of a solvent to be used. Stopper, and allow the container to stand at ordinary temperature with occasional stirring for about 5 days or until the soluble constituents have satisfactorily dissolved. Filter the liquid through cloth. In the case where about three-fourths of the total volume of the solvent is added, wash the residue with a suitable quantity of the solvent, press, and combine the filtrate and washings to make up the volume. In the case where the total volume of the solvent is added, sufficient solvent may be added, if necessary, to make up for the decreasing amount. Allow the mixture to stand for about 2 days, and obtain a clear liquid by decantation or filtration.

Percolation: Pour the solvent in small portions on crude drugs placed in a container, and mix well to moisten the crude drugs. Stopper the container, and allow it to stand for about 2 hours at room temperature. Pack the contents as tightly as possible in a suitable percolator, open the lower opening, and slowly pour sufficient solvent to cover the crude drugs. When the percolate begins to drip, close the opening, and allow the mixture to stand for 2 to 3 days at room temperature. Open the opening, and allow the percolate to drip at a rate of 1 to 3 mL per minute. Add an appropriate quantity of the solvent, and continue to percolate until the desired volume has passed. Mix thoroughly, allow standing for 2 days, and obtain a clear liquid by decantation or filtration. The time of standing and the flow rate may be varied depending on the kind and amount of crude drugs to be percolated.

Tinctures prepared by either of the above methods for which the content of the drug substance is specified are prepared by assaying the drug substance using a portion of the sample and adjusting, if necessary, with the percolate or with the solvent to the specified content.

GENERAL TESTS, PROCESSES 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, the procedures for acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, ammonium determination, arsenic determination, atomic absorption spectrophotometry, test for bacterial endotoxins, boiling point determination, distilling range determination, chloride determination, conductivity measurement, congealing point determination, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, endpoint detection in titrimetry, test of extractable volume for injection, flame coloration, fluorometry, foreign insoluble matter test for injections, foreign insoluble matter test for ophthalmic solutions, gas chromatography, heavy metals determination, test for glass containers for injections, 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, melting point determination, test for metal particles in ophthalmic ointments, methanol determination, microbial assay for antibiotics, test for microbial limit, test for microbial limit for crude drugs, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolarity determination, oxygen flask combustion method, particle size distribution test for preparations, pH determination, test for plastic containers, powder particle density determination, powder particle size determination, test for pyrogen, qualitative test, test for readily carbonizable substances, refractive index determination, residual solvents test, residue on ignition determination, test for rubber closure for aqueous infusions, specific gravity and density determination, specific surface area determination, test for sterility, sulfate determination, thermal analysis, thin-layer chromatography, test for total organic carbon, ultravioletvisible 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.

1.09 Qualitative Tests

Add the following next to Mercurous salt:

Mesilate

(1) To mesilates add twice its mass of sodium hydroxide, heat gently to melt, and continue heating for 20 to 30 seconds. After cooling, add a little amount of water, then add dilute hydrochloric acid, and warm: the gas evolved changes moistened potassium iodate-starch paper to blue.

(2) To mesilates add threefold its mass of sodium nitrate and anhydrous sodium carbonate, mix, and heat gradually. After cooling, dissolve the residue in diluted hydrochloric acid (1 in 5), and filter if necessary. The filtrate yields a white precipitate upon addition of barium chloride TS.

2.01 Liquid Chromatography

Change to read:

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

The ratio k represents the mass distribution ratio k' in liquid chromatography.

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column (t_0: time measured from the time of injection of a

compound with $k=0$ to the time of elution at the peak maximum), and the retention time (t_R: time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R=(1+k)\ t_0$$

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.

Procedure

Fix the detector, column and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

Identification and purity test

Identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the sample solution with a standard solution which is prepared by diluting the sample solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on the relative response factor to the principal component.

Assay

(1) Internal standard method—In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

(2) Absolute calibration curve method—Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights

on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

Method for peak measuring

Generally, the following methods are used.

(1) Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on both sides of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

(2) Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

System suitability

System suitability is an integral part of analytical methods using chromatography, and is used to ensure that the performance of the chromatographic systems used is adequate for the analysis of the drug to be tested, as the suitability of the method for the evaluation of the quality of the drug was verified. System suitability test should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability must be prescribed in the test method of the drug. Sample analysis is not acceptable unless the requirements of system suitability have been met.

In system suitability test of the chromatographic systems, the evaluation of ''System performance'' and ''System repeatability'' is usually required. For 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 validation data when the suitability of the method for the evaluation of the quality of the drug was verified.

Point to consider on changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the packing material, column temperature, composition ratio of the mobile phase, composition of buffer solutions in the mobile phase, pH of the mobile phase, concentration of ion-pair forming agents in the mobile phase, ionic strength of the mobile phase, flow rate of the mobile phase, number and timing of mobile phase composition changes in gradient program, flow rate of mobile phase in gradient program, composition and flow rate of derivatizing reagents, and reaction time and chamber temperature in chemical reaction may be modified within the ranges in which the liquid chromatographic system used conforms to the requirements of system suitability.

Terminology

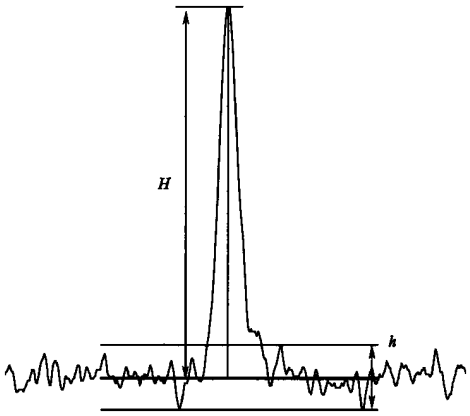
S/N ratio: It is defined by the following formula.

$$\mathrm{S/N} = \frac{2H}{h}$$

H: Peak height of the target ingredient peak from the baseline (the median value of background noise),

h: Width of background noise of the chromatogram of sample solution or solvent blank around the peak of the target ingredient.

The baseline and background noise are measured over a range 20 times of peak width at the center point of peak height of the target ingredient. When a solvent blank is used, measure over almost the same range as mentioned above around the point where the target ingredient elutes.



Symmetry factor: It shows the degree of symmetry of a peak in the chromatogram, and is defined as S in the following equation.

$$S = \frac{W_{0.05\,\mathrm{h}}}{2f}$$

$W_{0.05\,\mathrm{h}}$: Width of the peak at one-twentieth of the peak height,

f: Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height,

where $W_{0.05\,\mathrm{h}}$ and f have the same unit.

Relative standard deviation: Generally, it is defined as *RSD* (%) in the following equation.

$$RSD\ (\%) = \frac{100}{\bar{X}} \times \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{X})^2}{n-1}}$$

x_i: Observed value,

$\bar{X}$: Mean of observed values,

n: Number of replicate measurements.

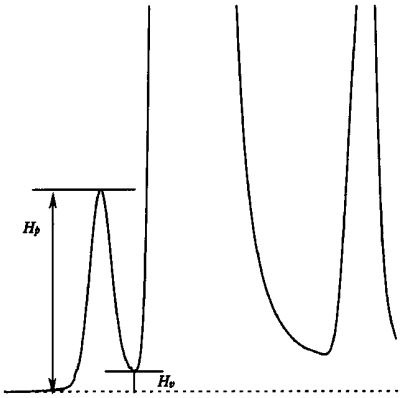
Complete separation of peak: It means that the resolution between two peaks is not less than 1.5. It is also called as ''baseline separation''.

Peak-valley ratio: It indicates the degree of separation between 2 peaks on a chromatogram when baseline separation cannot be attained, and is defined as p/v by the following formula.

$$p/v = \frac{H_p}{H_v}$$

H_p: peak height from the baseline of the minor peak,

H_v: height from the baseline of the lowest point (peak valley) of the curve between major and minor peaks.



Separation factor: It shows the relation between the retention times of peaks in the chromatogram, and is defined as α in the following equation.

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

t_{R1}, t_{R2}: Retention times of two compounds used for the resolution measurement ($t_{R1} < t_{R2}$),

t_0: Time of passage of the mobile phase through the column (time measured from the time of injection of a compound with $k = 0$ to the time of elution at the peak maximum).

The separation factor (α) indicates thermodynamic difference in partition of two compounds. It is basically the ratio of their partition equilibrium coefficients or of their mass-distribution ratios, and is obtained from the chromatogram as the ratio of the retention times of the two compounds.

Resolution: It shows the relation between the retention time and the peak width of peaks in the chromatogram, and is defined as R_S in the following equation.

$$R_S = 1.18 \times \frac{t_{R2} - t_{R1}}{W_{0.5\,h1} + W_{0.5\,h2}}$$

t_{R1}, t_{R2}: Retention times of two compounds used for the measurement of resolution ($t_{R1} < t_{R2}$),
$W_{0.5\,h1}$, $W_{0.5\,h2}$: Peak widths at half peak height,

where t_{R1}, t_{R2}, $W_{0.5\,h1}$ and $W_{0.5\,h2}$ have the same unit.

Number of theoretical plates: It indicates the extent of band broadening of a compound in the column, and is generally defined as N in the following equation.

$$N = 5.54 \times \frac{t_R^2}{W_{0.5\,h}^2}$$

t_R: Retention time of compound,
$W_{0.5\,h}$: Width of the peak at half peak height,

where t_R and $W_{0.5\,h}$ have the same unit.

Note Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

2.02 Gas Chromatography

Change to read:

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column (t_0: time measured from the time of injection of a compound with $k = 0$ to the time of elution at the peak maximum), and the retention time (t_R: time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k)t_0$$

Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram.

Identification and purity test

Identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the sample solution with a standard solution which is prepared by diluting the sample solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak

area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its relative response factor to the principal component.

Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

(1) Internal standard method—In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound.

(2) Absolute calibration curve method—Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the gas chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition.

(3) Standard addition method—Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area or peak height of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area or peak height on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

Method for peak measuring

Generally, the following methods are used.

(1) Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

(2) Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

System suitability

Refer to ''System suitability'' described under 2.01 Liquid Chromatography.

Point to consider in changing the operating conditions

Among the operating conditions specified in the individual monograph, inside diameter and length of column, particle size of packing material, concentration or thickness of stationary phase, column temperature, temperature rising-rate, kind and flow rate of carrier gas, and split ratio may be modified within the ranges in which the gas chromatographic system used conforms to the requirements of system suitability. Headspace sample injection device and its operating conditions may be also modified, provided that they give equivalent or more accuracy and precision.

Terminology

The definition of terms described under 2.01 Liquid Chromatography shall apply in 2.02 Gas Chromatography.

Note Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

2.48 Water Determination (Karl Fischer Method)

Change to read the following part under 1. Volumetric titration:

Preparation of test solutions and standard solutions

(1) Karl Fischer TS for water determination

Prepare according to the following method (i), (ii) or (iii). Additives may be added for the purpose of improving the stability or other performances if it is confirmed that they give almost the same results as those obtained from the specified method.

(i) Preparation 1

Dissolve 63 g of iodine in 100 mL of pyridine for Karl Fischer method, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding chloroform for Karl Fischer method or methanol for Karl Fischer method, and allow to stand for more than 24 hours before use.

(ii) Preparation 2

Dissolve 102 g of imidazole for Karl Fischer method in 350 mL of diethylene glycol monoethyl ether for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 64 g, keeping the temperature between 25°C and 30°C. Then dissolve 50 g of iodine in this solution, and allow to stand for more than 24 hours before use.

(iii) Preparation 3

Pass dried sulfur dioxide gas through 220 mL of propylene carbonate for water determination until the mass increase of the solvent reaches 32 g. To this solution, add 180 mL of propylene carbonate, or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for Karl Fischer method is dissolved and cooled in ice bath. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

These Karl Fischer TSs, prepared by any one of the above methods, must be standardized before every use, because of its activity change with the lapse of time. Further preserve the TS in a cold place, protecting it from light and moisture.

Standardization—According to the procedure described below, take a suitable quantity of methanol for Karl Fischer method in a dried titration flask, and titrate the solvent with a Karl Fischer TS to make the inside of the flask anhydrous. Then, weigh about 30 mg of water accurately and put it in the titration flask quickly, and titrate the water dissolved in the solvent with a Karl Fischer TS to the end point, under vigorous stirring. Calculate the water equivalence factor, f(mg/mL), corresponding to the amount of water (H_2O) in mg per 1 mL of the Karl Fischer TS by using the following equation:

$$f(\text{mg/mL}) = \frac{\text{Amount of water taken } (H_2O) \text{ (mg)}}{\text{Volume of Karl Fischer TS consumed for titration of water } (H_2O) \text{ (mL)}}$$

Change to read:

2.49 Optical Rotation Determination

Optical Rotation Determination is a method for the measurement of the angular rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In case of the ordinary light, the directions of the planes are unrestricted, while in case of the plane polarized light, commonly called as polarized light, the vibrations take place on only one plane that includes the advancing direction of the beam. And it is called that these beams have plane of polarization. Some drugs in the liquid state or in solution have a property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical constitution of the substance.

The optical rotation is a degree of rotation of polarized plane, caused by the optically active substance or its solution, and it is measured by the polarimeter. This value is proportional to the length of the polarimeter tube, and is also related to the solution concentration, the temperature and the measurement wavelength. The character of the rotation is indicated by the direction of the rotation, when facing to the advancing direction of the polarized light. Thus in case of rotation to the right, it is called dextrorotatory and expressed by placing plus sign (+), while in case of rotation to the left, it is called levorotatory and expressed by placing minus sign (−) before the figure of the angular rotation. For example, +20° means 20° of rotation to the right, while −20° means 20° of rotation to the left.

The angular rotation α_x^t means degree of rotation, when it

is measured at t°C by using specific monochromatic light x (expressed by wavelength of light source or the specific beam name). Usually, the measurement is performed at 20°C or 25°C, with a polarimeter tube of 100 mm in length, and with the D line of sodium lamp.

The specific rotation is expressed by the following equation:

$$[\alpha]_x^t = \frac{100\,\alpha}{lc}$$

t: The temperature of measurement.

x: The wavelength or the name of the specific monochromatic light (in the case of the Sodium D line, it is described as D).

α: The angle, in degrees, of rotation of the plane of the polarized light.

l: The thickness of the layer of sample solution, *i.e.*, the length of the polarimeter tube (mm).

c: Drug concentration in g/mL. When an intact liquid drug is used for the direct measurement without dilution by an appropriate solvent, c equals to its density (g/mL). However, unless otherwise specified, the specific gravity is conventionally used in stead of the density.

The description in the monograph, for example, "$[\alpha]_D^{20}$: -33.0 – $-36.0°$ (after drying, 1 g, water, 20 mL, 100 mm)," means the measured specific rotation $[\alpha]_D^{20}$ should be in the range of $-33.0°$ and $-36.0°$, when 1 g of accurately weighed sample dried under the conditions, specified in the test item of Loss on drying, is taken, and dissolved in water to make exactly 20 mL, then put in the polarimeter tube of 100 mm length, of which temperature is kept at 20°C.

4.01 Bacterial Endotoxins Test

Change to read the Preparation of Standard Endotoxin Stock Solution as follows:

Preparation of Standard Endotoxin Stock Solution

Prepare Standard Endotoxin Stock Solution by dissolving Endotoxin Reference Standard in water for bacterial endotoxins test (BET). Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

4.05 Microbial Limit Test

Change to read as follows:

4.05 Microbiological Examination of Non-sterile Products

This chapter includes microbial enumeration tests and tests for specified micro-organisms. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

I. Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

1 Introduction

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

2 General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any micro-organisms which are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

3 Enumeration Methods

Use the membrane filtration method, or the plate-count methods, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of micro-organisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

4 Growth Promotion Test and Suitability of the Counting Method

4-1 General considerations

The ability of the test to detect micro-organisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the

Table 4.05-I-1 Preparation and use of test micro-organisms

Micro-organism	Preparation of test strain	Growth promotion		Suitability of counting method in the presence of the product	
		Total aerobic microbial count	Total yeasts and moulds count	Total aerobic microbial count	Total yeasts and moulds count
Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276	*Casein soya bean digest agar or casein soya bean digest broth* 30 – 35°C 18 – 24 h	*Casein soya bean digest agar and casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days		*Casein soya bean digest agar/* MPN *casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days	
Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	*Casein soya bean digest agar* or *casein soya bean digest broth* 30 – 35°C 18 – 24 h	*Casein soya bean digest agar* and *casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days		*Casein soya bean digest agar*/MPN *casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days	
Bacillus subtilis such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134	*Casein soya bean digest agar* or *casein soya bean digest broth* 30 – 35°C 18 – 24 h	*Casein soya bean digest agar* and *casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days		*Casein soya bean digest agar*/MPN *casein soya bean digest broth* ≦100 CFU 30 – 35°C ≦3 days	
Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594	*Sabouraud-dextrose agar or Sabouraud-dextrose broth* 20 – 25°C 2 – 3 days	*Casein soya bean digest agar* ≦100 CFU 30 – 35°C ≦5 days	*Sabouraud-dextrose agar* ≦100 CFU 20 – 25°C ≦5 days	*Casein soya bean digest agar* ≦100 CFU 30 – 35°C ≦5 days MPN: not applicable	*Sabouraud-dextrose agar* ≦100 CFU 20 – 25°C ≦5 days
Aspergillus niger such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455	*Sabouraud-dextrose agar or potato-dextrose* agar 20 – 25°C 5 – 7 days, or until good sporulation is achieved	*Casein soya bean digest agar* ≦100 CFU 30 – 35°C ≦5 days	*Sabouraud-dextrose agar* ≦100 CFU 20 – 25°C ≦5 days	*Casein soya bean digest agar* ≦100 CFU 30 – 35°C ≦5 days MPN: not applicable	*Sabouraud-dextrose agar* ≦100 CFU 20 – 25°C ≦5 days

test is introduced.

4-2 Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 4.05-I-1.

Use *buffered sodium chloride-peptone solution pH 7.0* or *phosphate buffer solution pH 7.2* to make test suspensions; to suspend *A. niger* spores, 0.05 per cent of polysorbate 80 may be added to the buffer. Use the suspensions within 2 h or within 24 h if stored at 2 – 8°C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *A. niger* or *B. subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period of time.

4-3 Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms.

4-4 Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of *casein soya bean digest broth* and *casein soya bean digest agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate portion/plate of medium for

each. Inoculate plates of *Sabouraud-dextrose agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 4.05-I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

4-5 Suitability of the counting method in the presence of product

4-5-1 Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

Water-soluble products—Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in *buffered sodium chloride-peptone solution pH 7.0, phosphate buffer solution pH 7.2* or *casein soya bean digest broth*. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

Non-fatty products insoluble in water—Suspend the product to be examined (usually a 1 in 10 dilution is prepared) in *buffered sodium chloride-peptone solution pH 7.0, phosphate buffer solution pH 7.2* or *casein soya bean digest broth*. A surface-active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary adjust to pH 6 – 8. Further dilutions, where necessary, are prepared with the same diluent.

Fatty products—Dissolve in isopropyl myristate, sterilised by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent, heated if necessary to not more than 40°C, or in exceptional cases to not more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of the original product. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial tenfold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

Fluids or solids in aerosol form—Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

Transdermal patches—Remove the protective cover sheets (''release liner'') of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 min.

4-5-2 Inoculation and dilution

Add to the sample prepared as described above (4-5-1) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed.

If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

4-5-3 Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 4-5-2 and incubated following the procedure described in 4-5-4, is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluent, (3) membrane filtration or (4) a combination of the above measures.

Neutralizing agents—Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 4.05-I-2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizer and without product.

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the article is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

4-5-4 Recovery of micro-organism in the presence of product

For each of the micro-organisms listed in Table 4.05-I-1, separate tests are performed. Only micro-organisms of the added test strain are counted.

Table 4.05-I-2 Common neutralizing agents/method for interfering substances

Interfering substance	Potential neutralizing agents/method
Glutaraldehyde, Mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, Alcohol, Aldehydes, Sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), Parahydroxybenzoates (Parabens), Bis-biguanides	Lecithin
QAC, Parabens, Iodine	Polysorbate
Mercurials	Thioglycollate
Mercurials, Halogens, Aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

4-5-4-1 Membrane filtration

Use membrane filters having a nominal pore size not greater than 0.45 μm. The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed in Table 4.05-I-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under 4-5-1 to 4-5-3 (preferably representing 1 g of the product, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of *casein soya bean digest agar*. For the determination of total combined yeasts/moulds count (TYMC) transfer the membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plates as indicated in Table 4.05-I-1. Perform the counting.

4-5-4-2 Plate-count methods

Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

4-5-4-2-1 Pour-plate method

For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 4-5-1 to 4-5-3 and 15 – 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar*, both media being at not more than 45°C. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 4.05-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

4-5-4-2-2 Surface-spread method

For Petri dishes 9 cm in diameter, add 15 – 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar* at about 45°C to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-airflow cabinet or in an incubator. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under 4-5-1 to 4-5-3 over the surface of the medium. Incubate and count as prescribed under 4-5-4-2-1.

4-5-4-3 Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 4-5-1 to 4-5-3. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3 tubes with 9 – 10 mL of *casein soya bean digest broth*. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at 30 – 35°C for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or *casein soya bean digest agar*, for 1 – 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

4-6 Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in 4-5-2 in the absence of the product must be obtained. When verifying the

suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

5 Testing of Products

5-1 Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g. tablet, capsule, injection) is less than or equal to 1 mg or the amount per gram or millilitre (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product.

For materials used as active substances where sample quantity is limited or batch size is extremely small (i.e. less than 1000 mL or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorised.

For products where the total number of entities in a batch is less than 200 (e.g. samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

5-2 Examination of the product

5-2-1 Membrane filtration

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in section 4 and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of *casein soya bean digest agar*. For the determination of TYMC, transfer the other membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plate of *casein soya bean digest agar* at 30 – 35°C for 3 – 5 days and the plate of *Sabouraud-dextrose agar* at 20 – 25°C for 5 – 7 days. Calculate the number of CFU per gram or per millilitre of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under 4-5-1 separately through each of 2 sterile filter membranes. Transfer one membrane to *casein soya bean digest agar* for TAMC and the other membrane to *Sabouraud-dextrose agar* for TYMC.

5-2-2 Plate-count methods

5-2-2-1 Pour-plate method

Prepare the sample using a method that has been shown to be suitable as described in section 4. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of *casein soya bean digest agar* at 30 – 35°C for 3 – 5 days and the plates of *Sabouraud-dextrose agar* at 20 – 25°C for 5 – 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per millilitre of product.

5-2-2-2 Surface-spread method

Prepare the sample using a method that has been shown to be suitable as described in section 4. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

5-2-2-3 Most-probable-number method

Prepare and dilute the sample using a method that has been shown to be suitable as described in section 4. Incubate all tubes for 3 – 5 days at 30 – 35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per gram or millilitre of the product to be examined from Table 4.05-I-3.

5-3 Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using *casein soya bean digest agar*; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using *Sabouraud-dextrose agar*; if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, *Sabouraud-dextrose agar* containing antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

– 10^1 CFU: maximum acceptable count = 20,

– 10^2 CFU: maximum acceptable count = 200,

– 10^3 CFU: maximum acceptable count = 2000, and so forth.

The recommended solutions and media are described in *Tests for specified micro-organisms*.

II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

1 Introduction

The tests described hereafter will allow determination of

Table 4.05-I-3 Most-probable-number values of micro-organisms

Observed combinations of numbers of tubes showing growth in each set			MPN per g or per mL of product	95 per cent confidence limits
Number of g or mL of product per tube				
0.1	0.01	0.001		
0	0	0	Less than 3	0 – 9.4
0	0	1	3	0.1 – 9.5
0	1	0	3	0.1 – 10
0	1	1	6.1	1.2 – 17
0	2	0	6.2	1.2 – 17
0	3	0	9.4	3.5 – 35
1	0	0	3.6	0.2 – 17
1	0	1	7.2	1.2 – 17
1	0	2	11	4 – 35
1	1	0	7.4	1.3 – 20
1	1	1	11	4 – 35
1	2	0	11	4 – 35
1	2	1	15	5 – 38
1	3	0	16	5 – 38
2	0	0	9.2	1.5 – 35
2	0	1	14	4 – 35
2	0	2	20	5 – 38
2	1	0	15	4 – 38
2	1	1	20	5 – 38
2	1	2	27	9 – 94
2	2	0	21	5 – 40
2	2	1	28	9 – 94
2	2	2	35	9 – 94
2	3	0	29	9 – 94
2	3	1	36	9 – 94
3	0	0	23	5 – 94
3	0	1	38	9 – 104
3	0	2	64	16 – 181
3	1	0	43	9 – 181
3	1	1	75	17 – 199
3	1	2	120	30 – 360
3	1	3	160	30 – 380
3	2	0	93	18 – 360
3	2	1	150	30 – 380
3	2	2	210	30 – 400
3	2	3	290	90 – 990
3	3	0	240	40 – 990
3	3	1	460	90 – 1980
3	3	2	1100	200 – 4000
3	3	3	More than 1100	

the absence of, or limited occurrence of specified micro-organisms which may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results

as stated below.

Alternative microbiological procedures, including automated methods may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

2 General Procedures

The preparation of samples is carried out as described in *Microbial enumeration tests*.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in *Microbial enumeration tests*.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in *Microbial enumeration tests*.

3 Growth Promoting and Inhibitory Properties of the Media and Suitability of the Test

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

3-1 Preparation of test strains

Use standardised stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

3-1-1 Aerobic micro-organisms

Grow each of the bacterial test strains separately in containers containing *casein soya bean digest broth* or on *casein soya bean digest agar* at 30 – 35°C for 18 – 24 hours. Grow the test strain for *Candida albicans* separately on *Sabouraud-dextrose agar* or in *Sabouraud-dextrose broth* at 20 – 25°C for 2–3 days.

Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276,

Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

Escherichia coli such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

Salmonella enterica subsp. *enterica* serovar Typhimurium such as ATCC 14028

or, as an alternative,

Salmonella enterica subsp. *enterica* serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39,

Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594.

Use *buffered sodium chloride-peptone solution pH 7.0* or *phosphate buffer solution pH 7.2* to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 – 8°C.

3-1-2 Clostridia

Use *Clostridium sporogenes* such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under anaerobic conditions in *reinforced medium for Clostridia* at 30 – 35°C for 24 – 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Cl. sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 – 8°C for a validated period.

3-2 Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms.

3-3 Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.05-II-1.

Test for growth promoting *properties, liquid media*: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for growth promoting *properties, solid media*: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

Test for indicative properties: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

3-4 Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 4. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 4 using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in section 4.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see 4-5-3 of *Microbial Enumeration Tests*).

Table 4.05-II-1 Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
Test for bile-tolerant gram-negative bacteria		
Enterobacteria enrichment broth-Mossel	Growth promoting	*E. coli* *P. aeruginosa*
	Inhibitory	*S. aureus*
Violet red bile glucose agar	Growth promoting + Indicative	*E. coli* *P. aeruginosa*
Test for *Escherichia coli*		
MacConkey broth	Growth promoting	*E. coli*
	Inhibitory	*S. aureus*
MacConkey agar	Growth promoting + Indicative	*E. coli*
Test for *Salmonella*		
Rappaport Vassiliadis Salmonella enrichment broth	Growth promoting	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
	Inhibitory	*S. aureus*
Xylose, lysine, deoxycholate agar	Growth promoting + Indicative	*Salmonella enterica* subsp. *enterica* serovar Typhimurium or *Salmonella enterica* subsp. *enterica* serovar Abony
	Indicative	*E. coli*
Test for *Pseudomonas aeruginosa*		
Cetrimide agar	Growth promoting	*P. aeruginosa*
	Inhibitory	*E. coli*
Test for *Staphylococcus aureus*		
Mannitol salt agar	Growth promoting + Indicative	*S. aureus*
	Inhibitory	*E. coli*
Test for Clostridia		
Reinforced medium for Clostridia	Growth promoting	*Cl. sporogenes*
Columbia agar	Growth promoting	*Cl. sporogenes*
Test for *Candida albicans*		
Sabouraud dextrose broth	Growth promoting	*C. albicans*
Sabouraud dextrose agar	Growth promoting + Indicative	*C. albicans*

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralised, then it is to be assumed that the inhibited micro-organism will not be present in the product.

4 Testing of Products

4-1 Bile-tolerant gram-negative bacteria

4-1-1 Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1

Table 4.05-II-2 Interpretation of results

Results for each quantity of product			Probable number of bacteria per gram or mL of product
0.1 g or 0.1 mL	**0.01 g or 0.01 mL**	**0.001 g or 0.001 mL**	
+	+	+	more than 10^3
+	+	−	less than 10^3 and more than 10^2
+	−	−	less than 10^2 and more than 10
−	−	−	less than 10

g of the product to be examined as described in *Microbial enumeration tests*, but using *casein soya bean digest broth* as the chosen diluent, mix and incubate at 20 – 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

4-1-2 Test for absence

Unless otherwise prescribed use the volume corresponding to 1 g of the product, as prepared in 4-1-1 to inoculate *enterobacteria enrichment broth-Mossel*. Incubate at 30 – 35°C for 24 – 48 hours. Subculture on plates of *violet red bile glucose agar*. Incubate at 30 – 35°C for 18 – 24 hours.

The product complies with the test if there is no growth of colonies.

4-1-3 Quantitative test

4-1-3-1 Selection and subculture

Inoculate suitable quantities of *enterobacteria enrichment broth-Mossel* with the preparation as described under 4-1-1 and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of the product to be examined. Incubate at 30 – 35°C for 24 – 48 hours. Subculture each of the cultures on a plate of *violet red bile glucose agar*. Incubate at 30 – 35°C for 18 – 24 hours.

4-1-3-2 Interpretation

Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 4.05-II-2 the probable number of bacteria.

4-2 *Escherichia coli*

4-2-1 Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 3-4) of *casein soya bean digest broth*, mix and incubate at 30 – 35°C for 18 – 24 hours.

4-2-2 Selection and subculture

Shake the container, transfer 1 mL of *casein soya bean digest broth* to 100 mL of *MacConkey broth* and incubate at 42 – 44°C for 24 – 48 hours. Subculture on a plate of *MacConkey agar* at 30 – 35°C for 18 – 72 hours.

4-2-3 Interpretation

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

4-3 *Salmonella*

4-3-1 Sample preparation and pre-incubation

Prepare the product to be examined as described in *Microbial enumeration tests* and use the quantity corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under 3-4) of *casein soya bean digest broth*, mix and incubate at 30 – 35°C for 18 – 24 hours.

4-3-2 Selection and subculture

Transfer 0.1 mL of *casein soya bean digest broth* to 10 mL of *Rappaport Vassiliadis Salmonella enrichment broth* and incubate at 30 – 35°C for 18 – 24 hours. Subculture on plates of *xylose, lysine, deoxycholate agar*. Incubate at 30 – 35°C for 18 – 48 hours.

4-3-3 Interpretation

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

4-4 *Pseudomonas aeruginosa*

4-4-1 Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 3-4) of *casein soya bean digest broth* and mix. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in *Microbial enumeration tests* (4-5-1) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30 – 35°C for 18 – 24 hours.

4-4-2 Selection and subculture

Subculture on a plate of *cetrimide agar* and incubate at 30 – 35°C for 18 – 72 hours.

4-4-3 Interpretation

Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

4-5 *Staphylococcus aureus*

4-5-1 Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 3-4) of *casein soya bean digest broth* and homogenise. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in *Microbial enumeration tests* (4-5-1) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30 – 35°C for 18 – 24 hours.

4-5-2 Selection and subculture

Subculture on a plate of *mannitol salt agar* and incubate at 30 – 35°C for 18 – 72 hours.

4-5-3 Interpretation

The possible presence of *S. aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

4-6 Clostridia

4-6-1 Sample preparation and heat treatment

Prepare the product to be examined as described in *Microbial enumeration tests*.

Take 2 equal portions corresponding to not less than 1 g or 1 mL of the product to be examined. Heat 1 portion at 80°C for 10 min and cool rapidly. Do not heat the other portion.

4-6-2 Selection and subculture

Transfer the quantity corresponding to 1 g or 1 mL of the product to be examined from each of the mixed portions to 2 containers (38 mm × 200 mm) or other containers containing 100 mL of *reinforced medium for Clostridia*. Incubate under anaerobic conditions at 30 – 35°C for 48 hours. After incubation, make subcultures from each tube on *Columbia agar* and incubate under anaerobic conditions at 30 – 35°C for 48 hours.

4-6-3 Interpretation

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of Clostridia.

If no anaerobic growth of micro-organisms is detected on *Columbia agar* or the catalase test is positive, the product complies with the test.

4-7 *Candida albicans*

4-7-1 Sample preparation and pre-incubation

Prepare the product to be examined as described in *Microbial enumeration tests* and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL to inoculate 100 mL of *Sabouraud-dextrose broth* and mix. Incubate at 30 – 35°C for 3-5 days.

4-7-2 Selection and subculture

Subculture on a plate of *Sabouraud-dextrose agar* and incubate at 30 – 35°C for 24 – 48 hours.

4-7-3 Interpretation

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.

The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

The following section is given for information.

5 Recommended Solutions and Culture Media

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used if they have similar growth promoting and inhibitory properties.

Stock buffer solution. Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500 mL of purified water, adjust to pH 7.2 ± 0.2 with sodium hydroxide, add purified water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 – 8°C.

Phosphate buffer solution pH 7.2

Prepare a mixture of purified water and stock buffer solution (800:1 *V/V*) and sterilize.

Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g equivalent to 0.067 mol phosphate
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Purified water	1000 mL

Sterilize in an autoclave using a validated cycle.

Casein soya bean digest broth

Pancreatic digest of casein	17.0 g
Papaic digest of soya bean	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Casein soya bean digest agar

Pancreatic digest of casein	15.0 g
Papaic digest of soya bean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Sabouraud-dextrose agar

Glucose	40.0 g
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Agar	15.0 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Potato dextrose agar

Infusion from potatoes	200 g
Glucose	20.0 g
Agar	15.0 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Sabouraud-dextrose broth

Glucose	20.0 g
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Purified water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Enterobacteria enrichment broth-Mossel

Pancreatic digest of gelatin	10.0 g
Glucose monohydrate	5.0 g
Dehydrated ox bile	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dihydrate	8.0 g
Brilliant green	15 mg
Purified water	1000 mL

Adjust the pH so that after heating it is 7.2 ± 0.2 at 25°C. Heat at 100°C for 30 min and cool immediately.

Violet red bile glucose agar

Yeast extract	3.0 g
Pancreatic digest of gelatin	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Purified water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°C. Heat to boiling; do not heat in an autoclave.

MacConkey broth

Pancreatic digest of gelatin	20.0 g
Lactose monohydrate	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Purified water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

MacConkey agar

Pancreatic digest of gelatin	17.0 g
Peptones (meat and casein)	3.0 g
Lactose monohydrate	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30 mg
Crystal violet	1 mg
Purified water	1000 mL

Adjust the pH so that after sterilization it is 7.1 ± 0.2 at 25°C. Boil for 1 min with constant shaking then sterilize in an autoclave using a validated cycle.

Rappaport Vassiliadis Salmonella enrichment broth

Soya peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dipotassium hydrogen phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	36 mg
Purified water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°C. The pH is to be 5.2 ± 0.2 at 25°C after heating and autoclaving.

Xylose, lysine, deoxycholate agar

Xylose	3.5 g
L-Lysine	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium deoxycholate	2.5 g
Sodium thiosulfate	6.8 g
Ammonium iron (III) citrate	0.8 g
Purified water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°C. Heat to boiling, cool to 50°C and pour into Petri dishes. Do not heat in an autoclave.

Cetrimide agar

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified water	1000 mL
Glycerol	10.0 mL

Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilization it is 7.2 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Mannitol salt agar

Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g

Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Purified water	1000 mL

Heat to boiling for 1 min with shaking. Adjust the pH so that after sterilization it is 7.4 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Reinforced medium for Clostridia

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose monohydrate	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	0.5 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is about 6.8 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

Columbia agar

Pancreatic digest of casein	10.0 g
Meat peptic digest	5.0 g
Heart pancreatic digest	3.0 g
Yeast extract	5.0 g
Corn starch	1.0 g
Sodium chloride	5.0 g
Agar, according to gelling power	10.0 g to 15.0 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle. Allow to cool to 45 – 50°C; add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

6.01 Test for Metal Particles in Ophthalmic Ointments

Change the Preparation of test sample to read:

Preparation of test sample

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude 5 g each of their contents into separate flat-bottomed petri dishes 60 mm in diameter. Cover the dishes, and heat between 85°C and 110°C for 2 hours to dissolve bases completely. Allow the samples to cool to room temperature without agitation to solidify the contents. When the amount of the content is 5 g or less, extrude the contents as completely as practicable, and proceed in the same manner as described above.

Add the following next to Procedure:

Evaluation

The preparation complies with the test if the total number of metal particles of a size equal to or greater than 50 μm found in 10 units tested, is not more than 50, and also the number of dishes containing more than 8 particles is not more than 1. If this requirement is snot met, repeat the test with a further 20 units in the same manner, and if the total number of the particles found in the total of 30 units is not more than 150, and also the number of dishes containing more than 8 particles is not more than 3, the preparation complies with the test.

6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions

Add the following next to Procedure:

Evaluation

The preparation complies with the test if the calculated number per mL of insoluble particles of a size equal to or greater than 300 μm is not more than 1.

6.10 Dissolution Test

Change the Apparatus for Paddle Method (Apparatus 2) to read:

Apparatus for Paddle Method (Apparatus 2)—Use the assembly from Apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in Fig. 6.10-2. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of nonreactive material, such as not more than a few turns of wire helix or such one shown in Fig. 6.10-2a, may be attached to the dosage unit that would otherwise float. Other validated sinker devices may also be used. ◆If the use of sinker is specified, unless otherwise specified, use the sinker device shown in Fig. 6.10-2a.◆

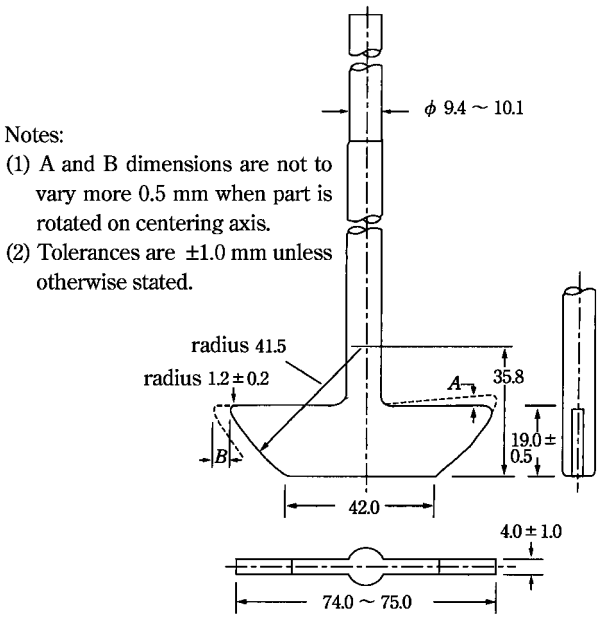


Fig. 6. 10-2 Apparatus 2, Paddle stirring element

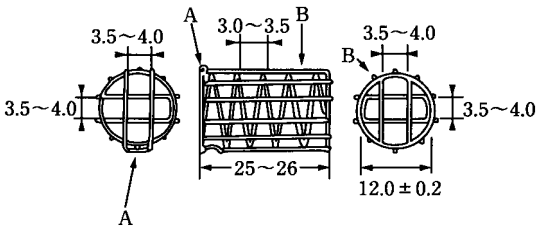


Fig. 6. 10-2a Alternative sinker

Add the following:

6.11 Foreign Insoluble Matter Test for Ophthalmic Solutions

Foreign Insoluble Matter Test for Ophthalmic Solutions is a test method to examine foreign insoluble matters in ophthalmic solutions.

When inspect with the unaided eyes at a position of luminous intensity of 3000 - 5000 lx under an incandescent lamp after cleaning the exterior of containers, Ophthalmic Solutions must be clear and free from readily detectable foreign insoluble matters.

9.01 Reference Standards

Change to read:

Reference Standards are the reference substances prepared to a specified quality necessary with regard to their intended use as prescribed in monographs of the Pharmacopoeia.

The Japanese Pharmacopoeia Reference Standards are as follows:

* A: Assay
AF: Anti-factor IIa activity
B: Bacterial Endotoxins Test <*4.01*>
C: Content ratio of active principle
D: Dissolution
DG: Digestion Test <*4.03*>
HB: Heparin-binding capacity
I: Identification
IS: Isomer ratio
M: Melting Point Determination <*2.60*>
P: Purity
T: Thermal Analysis <*2.52*>
U: Uniformity of dosage units
V: Vitamin A Assay <*2.55*>

(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*
Aceglutamide	I, P, A
Acetaminophen	I, A
Adrenaline Bitartrate	P
Alprostadil	I, P, A
p-Aminobenzoyl Glutamic Acid	P
Amitriptyline Hydrochloride	I, U, D, A
Amlexanox	I, U, D, A
Amlodipine Besilate	I, A
Anhydrous Lactose	I
Ascorbic Acid	A
Aspirin	A
Atropine Sulfate	I, A
Azathioprine	I, A
Baclofen	I, U, D, A
Baicalin	I, A
Beclometasone Dipropionate	I, A
Berberine Chloride	I, A
Betamethasone	I, P, U, D, A
Betamethasone Sodium Phosphate	I, A
Betamethasone Valerate	I, A
Bisacodyl	I, U, A
Caffeine	A
Calcium Folinate	I, A
Calcium Oxalate Monohydrate	T
Camostat Mesilate	I, A

d-Camphor	A
dl-Camphor	A
Carbidopa	I, P, A
Cellacefate	I
Chlordiazepoxide	I, P, U, A
Chlormadinone Acetate	I, A
Chlorpheniramine Maleate	I, U, A
Cholecalciferol	I, A
Ciclosporin	I, P, A
Cilostazol	I, U, D, A
Cisplatin	I, A
Clobetasol Propionate	I, A
Clofibrate	I, A
Clomifene Citrate	I, A
Cortisone Acetate	I, A
Cyanocobalamin	I, P, A
Deferoxamine Mesilate	I, A
Deslanoside	I, P, A
Dexamethasone	I, A
Diclofenamide	I, P, D, A
Diethylcarbamazine Citrate	A
Digitoxin	I, U, D, A
Digoxin	I, U, D, A
Dihydroergotoxine Mesilate	A
Dobutamine Hydrochloride	I, A
Edrophonium Chloride	I, A
Elcatonin	A
Enalapril Maleate	I, U, D, A
Endotoxin	B
Epitiostanol	P, A
Ergocalciferol	I, A
Ergometrine Maleate	P, U, A
Estradiol Benzoate	I, P, A
Estriol	I, U, D, A
Ethenzamide	A
Ethinylestradiol	I, U, D, A
Ethyl Aminobenzoate	A
Ethyl Icosapentate	I, P, A
Etoposide	I, A
Fluocinolone Acetonide	I, A
Fluocinonide	I, A
Fluorometholone	I, A
Fluoxymesterone	I, A
Folic Acid	I, U, A
Furosemide	I, U, D, A
Fursultiamine Hydrochloride	I, A
Gabexate Mesilate	I, P, A
Ginsenoside Rb_1	I, A
Ginsenoside Rg_1	I, A
Gitoxin	P
Glycyrrhizinic Acid	I, A
Gonadorelin Acetate	I, A
Guaifenesin	I, A
Heparin Sodium	HB, A
High-molecular Mass Urokinase	A
Human Chorionic Gonadotrophin	A
Human Insulin	I, A
Human Menopausal Gonadotrophin	P, A
Hydrochlorothiazide	I, A
Hydrocortisone	I, P, A
Hydrocortisone Acetate	I, A
Hydrocortisone Sodium Phosphate	I, A
Hydrocortisone Succinate	I, A
Idoxuridine	I, A
Imipramine Hydrochloride	I, U, D, A
Indomethacin	I, P, U, D, A
Insulin	P, A
Interleukin-2	A
Isoflurane	I, P, A
Kallidinogenase	A
Lactose	I
Lactulose	P, A
Lanatoside C	I, P, U, D, A
Limaprost	P, A
Low-molecular Mass Heparin	AF, A
Loxoprofen	A
Lysozyme	A
Maltose	A
Manidipine Hydrochloride	I, U, D, A
Mecobalamin	I, A
Melting Point Standard-Acetanilide	M
Melting Point Standard-Acetophenetidine	M
Melting Point Standard-Caffeine	M
Melting Point Standard-Sulfanilamide	M
Melting Point Standard-Sulfapyridine	M
Melting Point Standard-Vanillin	M
Menatetrenone	I, P, A
Mestranol	I, A
Methotrexate	I, A
Methoxsalen	I, A
Methyldopa	I, U, A
Methylergometrine Maleate	I, U, D, A
Methylprednisolone Succinate	I, P, A
Methyltestosterone	I, U, A
Metildigoxin	I, A
Mexiletine Hydrochloride	I, P, A
Mizoribine	I, U, D, A
Nabumetone	I, D, A
Neostigmine Methylsulfate	I, A
Nicotinamide	I, A
Nicotinic Acid	I, A
Nilvadipine	I, U, D, A
Nizatidine	I, U, D, A
Noradrenaline Bitartrate	P, A
Norgestrel	I, U, D, A
Oxytocin	P, A
Ozagrel Sodium	I, A
Paeoniflorin	I, A
Pentobarbital	P, A
Perphenazine	I, U, D, A
Phytonadione	A
Potassium Sucrose Octasulfate	P, A
Povidone	I
Pravastatin 1,1,3,3-tetramethylbutylammonium	I, A

Prednisolone	I, U, D, A
Prednisolone Acetate	I, A
Prednisolone Succinate	I, A
Primidone	A
Probenecid	I, D, A
Prochlorperazine Maleate	I, A
Progesterone	I, A
Protamine Sulfate	P
Puerarin	I, A
Pyridoxine Hydrochloride	I, A
Ranitidine Hydrochloride	I, A
Reserpine	I, U, D, A
Retinol Acetate	I, V
Retinol Palmitate	I, V
Riboflavin	I, A
Ritodrine Hydrochloride	I, U, D, A
Roxatidine Acetate Hydrochloride	I, U, D, A
Saccharated Pepsin	A
Scopolamine Hydrobromide	I, A
Sennoside A	I, A
Sennoside B	A
Serum Gonadotrophin	A
Spironolactone	I, A
Sulfadiazine Silver	I, A
Swertiamarin	I, A
Testosterone Propionate	I, A
Thiamine Chloride Hydrochloride	I, P, A
Thiamylal	A
Thrombin	A
Tocopherol	I, P, A
Tocopherol Acetate	I, A
Tocopherol Nicotinate	I, A
Tocopherol Succinate	A
Tolazamide	I, A
Tolbutamide	D
Tolnaftate	I, A
Tranexamic Acid	I, P, D, A
Triamcinolone	I, A
Triamcinolone Acetonide	I, A
Trichlormethiazide	I, U, D, A
Trihexyphenidyl Hydrochloride	I, U, D, A
Tyrosine	A, DG
Ubidecarenone	I, A
Ulinastatin	A
Vasopressin	A
Vinblastine Sulfate	I, U, A
Vincristine Sulfate	I, A
Warfarin Potassium	I, U, A
Zidovudine	I, A

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

Reference Standard	Intended Use*
Aclarubicin	A
Actinomycin D	I, A
Amikacin Sulfate	I, A
Amoxicillin	I, A
Amphotericin B	I, P, A
Ampicillin	I, P, A
Arbekacin Sulfate	I, A
Aspoxicillin	I, P, A
Astromicin Sulfate	I, A
Azithromycin	I, A
Aztreonam	I, P, A
Bacampicillin Hydrochloride	I, A
Bacitracin	I, A
Bekanamycin Sulfate	I, A
Benzylpenicillin Potassium	I, A
Bleomycin A_2 Hydrochloride	A
Carumonam Sodium	I, P, A
Cefaclor	I, P, U, D, A
Cefadroxil	I, U, D, A
Cefalexin	A
Cefalotin Sodium	I, P, A
Cefapirin Sodium	I, A
Cefatrizine Propylene Glycolate	I, A
Cefazolin	P, A
Cefbuperazone	A
Cefcapene Pivoxil Hydrochloride	I, U, A
Cefdinir	I, P, D, A
Cefditoren Pivoxil	I, U, D, A
Cefepime Dihydrochloride	I, A
Cefixime	I, P, A
Cefmenoxime Hydrochloride	I, P, A
Cefmetazole	A
Cefminox Sodium	I, A
Cefodizime Sodium	I, P, A
Cefoperazone	A
Cefotaxime	P, A
Cefotetan	I, P, A
Cefotiam Hexetil Hydrochloride	I, P, IS, A
Cefotiam Hydrochloride	I, P, A
Cefozopran Hydrochloride	I, A
Cefpiramide	P, A
Cefpirome Sulfate	I, A
Cefpodoxime Proxetil	I, IS, A
Cefroxadine	I, A
Cefsulodin Sodium	I, P, A
Ceftazidime	I, A
Cefteram Pivoxil Mesitylene Sulfonate	A
Ceftibuten Hydrochloride	A
Ceftizoxime	P, A
Ceftriaxone Sodium	I, A
Cefuroxime Axetil	I, P, IS, A
Cefuroxime Sodium	I, A
Chloramphenicol	I, A
Chloramphenicol Palmitate	I, A
Chloramphenicol Succinate	A
Ciclacillin	I, A
Clarithromycin	I, P, U, D, A
Clindamycin Hydrochloride	I, U, D, A
Clindamycin Phosphate	I, P, A
Cloxacillin Sodium	I, P, A
Colistin Sodium Methanesulfonate	I, A

Colistin Sulfate	A
Cycloserine	I, A
Daunorubicin Hydrochloride	I, A
Demethylchlortetracycline Hydrochloride	I, P, A
Dibekacin Sulfate	I, P, A
Dicloxacillin Sodium	I, A
Diethanolammonium Fusidate	A
Doxorubicin Hydrochloride	I, A
Doxycycline Hydrochloride	I, A
Enviomycin Sulfate	A
Epirubicin Hydrochloride	I, P, A
Erythromycin	I, P, A
Faropenem Sodium	I, P, U, A
Flomoxef Triethylammonium	P, A
Fosfomycin Phenethylammonium	A
Fradiomycin Sulfate	I, A
Gentamicin Sulfate	I, A
Gramicidin	I, A
Griseofulvin	I, P, U, A
Idarubicin Hydrochloride	I, U, A
Imipenem	I, U, A
Isepamicin Sulfate	I, P, A
Josamycin	I, C, U, A
Josamycin Propionate	I, A
Kanamycin Monosulfate	I, P, A
Latamoxef Ammonium	P, A
Lenampicillin Hydrochloride	I, A
Leucomycin A_5	C, A
Lincomycin Hydrochloride	I, P, A
Lithium Clavulanate	A
Meropenem	I, A
Micronomicin Sulfate	I, A
Midecamycin	I, A
Midecamycin Acetate	I, A
Minocycline Hydrochloride	I, P, A
Mitomycin C	I, U, A
Mupirocin Lithium	P, A
Netilmicin Sulfate	I, A
Nystatin	I, P, A
Oxytetracycline Hydrochloride	I, A
Panipenem	A
Peplomycin Sulfate	I, A
Phenethicillin Potassium	A
Pimaricin	I, A
Piperacillin	I, A
Pirarubicin	I, P, A
Pivmecillinam Hydrochloride	I, P, A
Polymixin B Sulfate	I, A
Pyrrolnitrin	I, A
Ribostamycin Sulfate	I, A
Rifampicin	I, P, A
Rokitamycin	I, U, D, A
Roxithromycin	I, P, A
Siccanin	I, A
Sisomicin Sulfate	I, A
Spectinomycin Hydrochloride	I, A
Spiramycin Acetate II	C, A
Streptomycin Sulfate	I, A
Sulbactam	P, A
Sulbenicillin Sodium	I, A
Sultamicillin Tosilate	I, A
Talampicillin Hydrochloride	I, A
Teicoplanin	I, A
Tetracycline Hydrochloride	I, P, A
Tobramycin	I, A
Trichomycin	A
Vancomycin Hydrochloride	I, A
Zinostatin Stimalamer	I, A

9.21 Standard Solutions for Volumetric Analysis

Add the following:

Zinc sulfate, 0.02 mol/L

1000 mL of this solution contains 5.7516 g of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$: 287.58).

Preparation—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly 5 times the initial volume.

9.22 Standard Solutions

Add the following:

Standard Aluminum Solution for Atomic Absorption Spectrophotometry To exactly 10 mL of Standard Aluminum Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.100 mg of aluminum (Al).

Standard Iron Stock Solution Dissolve exactly 4.840 g of iron (III) chloride hexahydrate in diluted hydrochloric acid (9 in 25) to make exactly 100 mL.

Standard Iron Solution for Atomic Absorption Spectrophotometry To exactly 5 mL of Standard Iron Stock Solution add water to make exactly 200 mL. Prepare before use. Each mL of this solution contains 0.250 mg of iron (Fe).

Standard Magnesium Stock Solution Dissolve exactly 8.365 g of magnesium chloride hexahydrate in 2 mol/L hydrochloric acid TS to make exactly 1000 mL.

Standard Magnesium Solution for Atomic Absorption Spectrophotometry To exactly 1 mL of Standard Magnesium Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL of this solution contains 0.0100 mg of magnesium (Mg).

9.41 Reagents, Test Solutions

Change the following to read:

Albiflorin $C_{23}H_{28}O_{11}.xH_2O$ White powder having no odor. Freely soluble in water, in methanol and in ethanol

(99.5).

Identification Determine the absorption spectrum of a solution of albiflorin in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 230 nm and 234 nm.

Purity (1) Related substances 1—Dissolve 1 mg of albiflorin in 1 mL of mehthanol, and perform the test with 10 μL of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot which appears at around *R*f 0.2 does not appear.

(2) Related substances 2—Dissolve 1 mg of albiflorin in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Peony Root, and measure the peak areas about 2 times as long as the retention time of peoniflorin: the total area of the peaks other than albiflorin from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Amygdalin for thin-layer chromatography $C_{20}H_{27}NO_{11}$ A white, odorless powder. Soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification Determine the absorption spectrum of a solution of amygdalin for thin-layer chromatography in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, between 261 nm and 265 nm, and between 267 nm and 271 nm.

Purity Related substances—Dissolve 5 mg of amygdalin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Peach Kernel: any spot other than the principal spot at the *R*f value of about 0.3 obtained from the sample solution is not more intense than the spot from the standard solution.

Capsaicin for component determination See (*E*)-capsaicin for component determination.

Capsaicin for thin-layer chromatography See (*E*)-capsaicin for thin-layer chromatography.

Chlorogenic acid for thin-layer chromatography See (*E*)-chlorogenic acid for thin-layer chromatography.

Cinnamaldehyde for thin-layer chromatography See (*E*)-cinnamaldehyde for thin-layer chromatography.

Dehydrocorydaline nitrate for component determination $C_{22}H_{24}N_2O_7$ Yellow, crystals or crystalline powder. It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). *Melting point*: about 240°C (with decomposition).

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (333 nm): 577 – 642 (3 mg, water, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the test.

Purity (1) Related substances 1—Dissolve 5.0 mg of dehydrocorydaline nitrate for component determination in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Spray Dragendorff's TS on the plate, air-dry, and spray sodium nitrite TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(2) Related substances 2—Dissolve 5.0 mg of dehydrocorydaline nitrate for component determination in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of peaks other than dehydrocorydaline from the sample solution is not larger than the peak area of dehydrocorydaline 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 Corydalis Tuber.

Detector: Ultraviolet absorption photometer (wavelength: 230 nm)

Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline, beginning after the peak of nitric acid.

System suitability

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

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained from 5 μL of this solution is equivalent to 3.5 to 6.5% of that from 5 μL of the standard solution.

[6]-Gingerol for thin-layer chromatography $C_{17}H_{26}O_4$ A yellow-white to yellow, liquid or solid. Freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification Determine the absorption spectrum of a solution of [6]-gingerol for thin-layer chromatography in ethanol (99.5) (7 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 279 nm and 283 nm.

Purity Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of

methanol. Perform the test with 10 μL of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the *R*f value of about 0.3 does not appear.

Magnolol for component determination Use magnolol for thin-layer chromatography meeting the following additional specifications.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (290 nm): 270 – 293 (10 mg, methanol, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour for the sample.

Purity Related substances—Dissolve 5.0 mg of magnolol for component determination in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 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 area of each peak from these solutions by the automatic integration method: the total area of peaks other than the peak of magnolol from the sample solution is not larger than the peak area of magnolol 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 Magnolia Bark.

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

System suitability

System performance, and system repeatability: Proceed as directed in the system suitability in the component determination under Magnolia Bark.

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

Add the following:

Alminoprofen for assay $C_{13}H_{17}NO_2$ [Same as the monograph Alminoprofen. When dried, it contains not less than 99.5% of alminoprofen ($C_{13}H_{17}NO_2$).]

6-Amidino-2-naphthol methanesulfonate
$C_{11}H_{10}N_2O.CH_4O_3S$ A white to pale yellow crystalline powder. Melting point: about 233°C (with decomposition).

Purity A solution obtained by dissolving 0.5 g of 6-amidino-2-naphthol methanesulfonate in 10 mL of methanol is clear.

Aminopyrine $C_{13}H_{17}N_3O$ White to pale yellow crystals or crystalline powder.

Melting point ⟨*2.60*⟩: 107 – 109°C

Amosulalol hydrochloride for assay $C_{18}H_{24}N_2O_5S.HCl$ [Same as the monograph Amosulalol Hydrochloride. It contains not less than 99.0% of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), calculated on the anhydrous basis.]

Amygdalin for component determination Amygdalin for thin-layer chromatography. However, it meets the following requirements:

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (263 nm): 55 – 58 [20 mg, methanol, 20 mL; separately determine the water ⟨*2.48*⟩ (5 mg, coulometric titration) and calculate on the anhydrous basis].

Purity Related substances—Dissolve 5 mg of amygdalin for component determination in 10 mL of the mobile phase, and use this as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this 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 each peak area by the automatic integration method: the total area of the peaks other than amygdalin from the sample solution is not larger than the peak area of amygdalin 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 (3) under Keishibukuryogan Extract.

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

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 amygdalin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Keishibukuryogan Extract.

Bisoprolol fumarate for assay $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$ [Same as the monograph Bisoprolol Fumarate. However, when dried, it contains not less than 99.0% of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$]. Also, when performing the Purity (2) under Bisoprolol Fumarate, the total area of the peaks other than bisoprolol is not greater than 1/5 times the peak area of bisoprolol from the standard solution].

Purify as follows if needed.

Purification method—Dissolve, with heating, 2 g of Bisoprolol Fumarate in 200 mL of ethyl acetate, add 0.5 g of activated carbon, shake well, and filter using a glass filter (G4). Place the filtrate in ice water for 2 hours while occasional shaking. Collect the crystals that precipitate out using a glass filter (G3). Dry the crystals obtained in vacuum at 80°C for 5 hours using phosphorus (V) oxide as a dessicant.

Bromothymol blue-sodium hydroxide-ethanol TS Dissolve 50 mg of bromothymol blue in 4 mL of diluted 0.2 mol/L sodium hydroxide TS (1 in 10) and 20 mL of ethanol (99.5), and add water to make 100 mL.

Bucillamine for assay $C_7H_{13}NO_3S_2$ [Same as the monograph Bucillamine. However, when dried, it contains not

less than 99.0% of bucillamine ($C_7H_{13}NO_3S_2$). Furthermore, it conforms to the following test.]

Purity Related substances—Dissolve 60 mg of bucillamine for assay in 20 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 the mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. When the test is performed according to the Purity (3) under Bucillamine, the total area of the peaks other than the bucillamine peak from the sample solution is not larger than the peak area of bucillamine from the standard solution.

Buformin hydrochloride for assay $C_6H_{15}N_5.HCl$ [Same as the monograph Buformin Hydrochloride. When dried, it contains not less than 99.5% of buformin hydrochloride ($C_6H_{15}N_5.HCl$).]

Butyl benzoate $C_6H_5COOCH_2CH_2CH_2CH_3$ A clear and colorless liquid.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.495 – 1.500

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.006 – 1.013

***n*-Butylboronic acid** $C_4H_{11}BO_2$ White flakes.

Melting point ⟨*2.60*⟩: 90 – 92°C

(*E*)-Capsaicin for component determination Use (*E*)-capsaicin for thin-layer chromatography meeting the following additional specifications.

Absorbance ⟨*2.24*⟩ $E_{1\,cm}^{1\%}$ (281 nm): 97 – 105 (10 mg, methanol, 200 mL). Use the sample dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours for the test.

Purity Related substances—Dissolve 10 mg of capsaicin for component determination in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of the peaks other than capsaicin from the sample solution is not larger than the peak area of capsaicin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed the operating conditions in the Component determination under Capsicum.

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

System suitability

System performance, and system repeatability: Proceed the system suitability in the Component determination under Capsicum.

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 capsaicin from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of capsaicin from 20 μL of the standard solution.

(*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*⟩: 64.5 – 66.5°C

Purity Related substances—Dissolve 20 mg of capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 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.

Cesium chloride CsCl White crystals or crystalline powder. Very soluble in water, and freely soluble in ethanol (99.5).

Loss on drying ⟨*2.41*⟩: Not more than 1.0% (1 g, 110°C, 2 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.5 g, previously dried, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (indicator: fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 16.84 mg of CsCl

Cesium chloride TS To 25.34 g of cesium chloride add water to make 1000 mL.

Cetirizine hydrochloride for assay $C_{21}H_{25}ClN_2O_3.2HCl$ [Same as the monograph Cetirizine Hydrochloride. When dried, it contains not less than 99.5% of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$).]

3′-Chloro-3′-deoxythymidine for liquid chromatography $C_{10}H_{13}N_2O_4Cl$ Occurs as a white powder.

Purity—Dissolve 10 mg of 3′-chloro-3′-deoxythymidine for liquid chromatography in the mobile phase to make 100 mL. Perform the test with 10 μL of this solution as directed in the Purity **(3)** under Zidovudine: a peak is not observed at the retention time for zidovudine.

(*E*)-Chlorogenic acid for thin-layer chromatography $C_{16}H_{18}O_9.xH_2O$ A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution 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, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot at around *R*f 0.5 appears.

Chlorphenesin carbamate for assay $C_{10}H_{12}ClNO_4$ [Same as the monograph Chlorphenesin Carbamate. When dried, it contains not less than 99.0% of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$).]

Cibenzoline succinate for assay $C_{18}H_{18}N_2.C_4H_6O_4$ [Same as the monograph Cibenzoline Succinate. When dried, it contains not less than 99.0% of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) and meets the following requirement.]

Purity: Related substances—Dissolve 0.10 g in 2 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. To exactly 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 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, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution. On standing the plate for 30 minutes in the tank saturated with iodine vapor, the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Cilazapril $C_{22}H_{31}N_3O_5.H_2O$ [Same as the monograph Cilazapril Hydrate]

Cilazapril for assay $C_{22}H_{31}N_3O_5.H_2O$ [Same as the monograph Cilazapril Hydrate. It contains not less than 99.0% of cilazapril ($C_{22}H_{31}N_3O_5$), calculated on the anhydrous basis.]

(*E*)-Cinnamaldehyde for thin-layer chromatography C_9H_8O A colorless or light yellow liquid, having a characteristic aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (285 nm): 1679 – 1943 (5 mg, methanol, 2000 mL)

Purity Related substances—Dissolve 10 mg in 2 mL of methanol. Perform the test with 1 μL of this solution as directed in the Identification (3) under Kakkonto Extract: no spot other than the principal spot (*R*f value is about 0.4) appears.

Clorazepate dipotassium for assay $C_{16}H_{10}ClKN_2O_3.KOH$ [Same as the monograph Clorazepate Dipotassium. When dried it contains not less than 99.0% of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3.KOH$).]

1-[(2*R*,5*S*)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl] thymine for thin-layer chromatography $C_{10}H_{12}N_2O_4$ Occurs as a white powder.

Purity—Dissolve 0.1 g of 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity **(2)** under Zidovudine: spots other than the principal spot with an *R*f value of about 0.23 are not observed.

Dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS To 5 mL of a solution of sodium pentacyanonitrosylferrate (III) dihydrate (3 in 50), 5 mL of a solution of potassium hexacyanoferrate (III) (13 in 200) and 2.5 mL of a solution of sodium hydroxide (1 in 10) add water to make 25 mL, and mix. Use after the color of the solution changes from dark red to light yellow. Prepare at the time of use.

1,2-Dinitrobenzene $C_6H_4(NO_2)_2$ Occurs as yellowish white to brownish yellow crystals or a crystalline powder.

Identification: Determine the infrared absorption spectrum of 1,2-dinitrobenzene as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3100 cm^{-1}, 1585 cm^{-1}, 1526 cm^{-1}, 1352 cm^{-1}, and 793 cm^{-1}.

Melting point ⟨*2.60*⟩: 116 – 119°C

Enalapril maleate $C_{20}H_{28}N_2O_5.C_4H_4O_4$ [Same as the namesake monograph]

2-Ethylhexyl parahydroxybenzoate $C_{15}H_{22}O_3$ Pale yellow, clear viscous liquid. Miscible with methanol (99.5). Practically insoluble in water.

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
= 250.3 mg of $C_{15}H_{22}O_3$

Etizolam for assay $C_{17}H_{15}ClN_4S$ [Same as the monograph Etizolam. When dried, it contains not less than 99.0% of etizolam ($C_{17}H_{15}ClN_4S$).]

[6]-Gingerol for component determination [6]-Gingerol for thin-layer chromatography. However, it meets the following requirements:

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (281 nm): 101 – 112 [7 mg, ethanol (99.5), 200 mL].

Purity Related substances—Dissolve 5 mg of [6]-gingerol for component determination in 5 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this 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 each peak area by the automatic integration method: the total area of the peaks other than [6]-gingerol from the sample solution is not larger than the peak area of [6]-gingerol 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 (3) under Hangekobokuto Extract.

Time span of measurement: About 6 times as long as the

retention time of [6]-gingerol.

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 [6]-gingerol obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

4-Hydroxyisophthalic acid $HOC_6H_3(COOH)_2$ White crystals or powder.

Content: not less than 98.0%. Assay—Weigh accurately about 0.14 g, dissolve in 50 mL of ethanol (95), 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
= 9.106 mg of $C_8H_6O_5$

Hyperoside for thin-layer chromatography $C_{21}H_{20}O_{12}$ Yellow crystals or crystalline powder. Slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 220°C (with decomposition).

Identification: Determine the absorption spectrum of a solution of hyperoside for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 255 nm and 259 nm.

Purity Related substances—Dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Crataegus Fruit: any spot other than the principal spot of around *R*f 0.5 does not appear.

Isoxsuprine hydrochloride for assay $C_{18}H_{23}NO_3.HCl$ [Same as the monograph Isoxsuprine Hydrochloride]

Labetalol hydrochloride $C_{19}H_{24}N_2O_3.HCl$ [Same as the namesake monograph]

Labetalol hydrochloride for assay $C_{19}H_{24}N_2O_3.HCl$ [Same as the monograph Labetalol Hydrochloride. However, when dried, it contains not less than 99.0% of labetalol hydrochloride ($C_{19}H_{24}N_2O_3.HCl$).]

Lanthanum chloride TS To 58.65 g of lanthanum (III) oxide add 100 mL of hydrochloric acid, and boil. After cooling, add water to make 1000 mL.

Magnolol for thin-layer chromatography $C_{18}H_{18}O_2$ Odorless, white crystals or crystalline powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 102°C.

Identification: Determine the absorption spectrum of a solution of magnolol for thin-layer chromatography in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 287 nm and 291 nm.

Purity Related substances—Dissolve 1.0 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, acetone and acetic acid (100) (20:15:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of around *R*f 0.5 does not appear.

Miconazole nitrate $C_{18}H_{14}Cl_4N_2O.HNO_3$ [Same as the namesake monograph]

Minocycline hydrochloride $C_{23}H_{27}N_3O_7.HCl$ [Same as the namesake monograph]

Nile blue $C_{20}H_{20}ClN_3O$ Blue-green powder.

3-Nitroaniline $C_6H_6N_2O_2$ Yellow crystals or crystalline powder.

Melting point ⟨*2.60*⟩: 112 – 116°C

Nodakenin for thin-layer chromatography $C_{20}H_{24}O_9$ White powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 220°C (with decomposition).

Identification: Determine the absorption spectrum of a solution of nodakenin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 333 nm and 337 nm.

Optical rotation ⟨*2.49*⟩: $[\alpha]_D^{20}$: +50 – +68° (5 mg, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 1 mg of nodakenin for thin-layer chromatography in 3 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5 μL each of these solutions as directed in the Identification (2) under Peucedanum Root: the spot other than the principal spot of around *R*f 0.3 from the sample solution is not more intense than the spot from the standard solution.

Oleic acid $C_{18}H_{34}O_2$ Occurs as a colorless or pale yellow transparent liquid and has a slightly distinct odor. It is miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: about 0.9

Content: not less than 99.0%. Assay—To 40 μL of oleic acid to be examined add 1 mL of a solution of boron trifluoride in methanol (3 in 20), mix, and heat on a water bath for 3 minutes. After cooling, add 10 mL of petroleum ether and 10 mL of water, shake, collect the ether layer after allowing to stand, and use as the sample solution. Perform the test with 0.2 μL of the sample solution 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 methyl oleate by the area percentage method.

Operating conditions

Detector: A hydrogen flame-ionization detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (149 – 177 μm) coated with methyl polyacrylate in a rate of 5 – 10%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Helium

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

Time span of measurement: About 2 times as long as the retention time of methyl oleate, beginning after the solvent peak.

(±)-Praeruptorin A for thin-layer chromatography

$C_{21}H_{22}O_7$ White crystals or crystalline powder. Soluble in methanol, sparingly slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification: Determine the absorption spectrum of a solution of (±)-praeruptorin A for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*: it exhibits a maximum between 320 nm and 324 nm.

Melting point ⟨2.60⟩: 152 – 156°C

Purity Related substances—Dissolve 2 mg of (±)-praeruptorin A for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed with 5 μL each of these solutions as directed in the Identification (1) under Peucedanum Root: the spot other than the principal spot of around *R*f 0.3 from the sample solution is not more intense than the spot from the standard solution.

Rosmarinic acid for component determination Rosmarinic acid for thin-layer chromatography. However, it meets the following requirements:

Absorbance ⟨2.24⟩ $E_{1\,cm}^{1\%}$ (332 nm): 526 – 559 [5 mg, ethanol (99.5), 500 mL].

Purity Related substances—Dissolve 5 mg of rosmarinic acid for component determination in 20 mL of the mobile phase, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this 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 each peak area by the automatic integration method: the total area of the peaks other than rosmarinic acid from the sample solution is not larger than the peak area of rosmarinic acid 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 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of rosmarinic acid is about 14 minutes.

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

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 rosmarinic acid obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rosmarinic acid 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 rosmarinic acid is not more than 1.5%.

Rosmarinic acid for thin-layer chromatography

$C_{18}H_{16}O_8$ White to pale yellow crystals or crystalline powder. Freely soluble in ethanol (99.5), and slightly soluble in water. Melting point: about 205°C (with decomposition).

Identification: Determine the absorption spectrum of a solution of rosmarinic acid for thin-layer chromatography in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*: it exhibits maxima between 217 nm and 221 nm, between 290 nm and 294 nm, and between 330 nm and 334 nm.

Purity Related substances—Dissolve 10 mg of rosmarinic acid for thin-layer chromatography in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Proceed with 10 μL each of the sample solution and standard solution as directed in the Identification (2) under Hangekobokuto Extract: the spot other than the principal spot of around *R*f 0.5 from the sample solution is not more intense than the spot from the standard solution.

Sodium di-2-ethylhexyl sulfosuccinate

$C_8H_{17}COOCH_2(C_8H_{17}COO)CHSO_3Na$ White or translucent white mucilaginous soft masses. Sparingly soluble in water.

Purity Clarity and color of solution: A solution prepared by dissolving 1.0 g in 100 mL of water is clear and colorless.

Loss on drying ⟨2.41⟩: not more than 5.0% (1 g, 105°C, 2 hours).

Sodium dihydrogen phosphate TS, pH 2.2 Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 800 mL of water, adjust the pH to 2.2 with phosphoric acid, and add

water to make 1000 mL.

1 mol/L Sulfuric acid TS Add 60 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

5 mol/L Sulfuric acid TS Add 300 mL of sulfuric acid in 1000 mL of water slowly with stirring, then allow to cool.

Thymine for liquid chromatography $C_5H_6N_2O_2$ Occurs as a white powder.

Purity—Dissolve 10 mg of the substance to be examined in 100 mL of methanol, add the mobile phase to make exactly 250 mL, and use this solution as the sample solution. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μL each of these solutions and perform the test as directed in the Purity **(3)** under Zidovudine. Determine the area of each peak in the sample and standard solutions by the automatic integration method: the total area of peaks other than thymine from the sample solution is not larger than that from the standard solution. However, the time span of measurement is about 10 times the retention time of thymine, beginning after the solvent peak.

Thymol for spraying test solution $C_{10}H_{14}O$ White crystals or crystalline powder, having an aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification: Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 2960 cm^{-1}, 1420 cm^{-1}, 1290 cm^{-1}, 1090 cm^{-1} and 810 cm^{-1}.

Melting point ⟨*2.60*⟩: 49 – 52°C

Purity: Other phenols—Shake vigorously 1.0 g of the substance to be examined with 20 mL of warm water for 1 minute, and filter. To 5 mL of the filtrate add 1 drop of a solution of iron (III) chloride hexahydrate (27 in 100): the solution reveals a green but not a blue to purple color.

Thymol-sulfuric acid-methanol TS for spraying Dissolve 1.5 g of thymol for spraying test solution in 100 mL of methanol, and add 5.7 mL of sulfuric acid.

Triphenylmethanol for thin-layer chromatography $C_{19}H_{15}OH$ Occurs as a white powder.

Purity—Dissolve 0.1 g of triphenylmethanol for thin-layer chromatography in 100 mL of methanol and perform the test as directed in the Purity **(2)** under Zidovudine: spots other than the principal spot with an *R*f value of about 0.73 are not observed.

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

Porous styrene-divinylbenzene copolymer for liquid chromatography A porous styrene-divinylbenzen copolymer prepared for liquid chromatography.

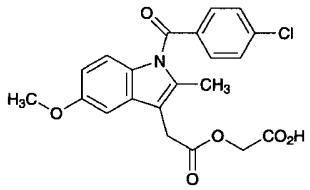
Strongly basic ion exchange resin for column chromatography Prepared for column chromatography.

Official Monographs

Add the following:

Acemetacin

アセメタシン



$C_{21}H_{18}ClNO_6$: 415.82
2-{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyloxy}acetic acid [*53164-05-9*]

Acemetacin, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{18}ClNO_6$.

Description Acemetacin occurs as a light yellow crystalline powder.

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

Identification **(1)** To 1 mg of Acemetacin add 1 mL of concentrated chromotropic acid TS, and heat in a water bath for 5 minutes: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Acemetacin in methanol (1 in 50,000) 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 Acemetacin as directed in the potassium bromide disk method under Infrared Spectrometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Acemetacin as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 151 – 154°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Acemetacin 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 0.40 g of Acemetacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 1 mL of this solution, add acetone 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 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): not more than 2 spots other than the principal spot appear from the sample solution, and these spots are not more intense than the spot obtained from the standard solution.

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 0.35 g of Acemetacin, previously dried, dissolve in 20 mL of acetone, add 10 mL of water, and then titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same method, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 41.58 mg of $C_{21}H_{18}ClNO_6$

Containers and storage Containers—Tight containers.

Acetylcholine Chloride for Injection

注射用アセチルコリン塩化物

Add the following next to Residue on ignition:

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.

Ajimaline Tablets

アジマリン錠

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 Ajimaline Tablets add 150 mL of 2nd fluid for dissolution test, shake to disintegrate the tablet, then add 2nd fluid for dissolution test to make exactly 200 mL, and filter this solution 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 equivalent to about 0.5 mg of ajimaline ($C_{20}H_{26}N_2O_2$), add 2nd fluid for dissolution test to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ajimaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in 2nd fluid for dissolution test to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

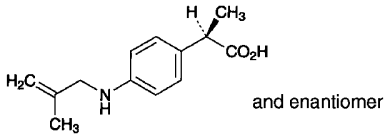
$$\text{Amount (mg) of ajimaline } (C_{20}H_{26}N_2O_2) = W_S \times (A_T/A_S) \times (1/V) \times 4$$

W_S: Amount (mg) of ajimaline for assay

Add the following:

Alminoprofen

アルミノプロフェン



$C_{13}H_{17}NO_2$: 219.28
(2*RS*)-2-{[4-(2-Methylprop-2-en-1-yl)amino]phenyl}propanoic acid [*39718-89-3*]

Alminoprofen, when dried, contains not less than 99.0% and not more than 101.0% of $C_{13}H_{17}NO_2$.

Description Alminoprofen occurs as white to pale yellow crystals or crystalline powder.

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

It gradually turns brown on exposure to light.

A solution of Alminoprofen in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Alminoprofen in ethanol (99.5) (3 in 500,000) 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 Alminoprofen 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*⟩ 106 – 108°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Alminoprofen 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 Alminoprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Alminoprofen in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 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 these solutions by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/5 times the peak area of alminoprofen from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than the peak area of alminoprofen from the standard solution.

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 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 1000) (4:1).

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

Time span of measurement: About 5 times as long as the retention time of alminoprofen, 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 alminoprofen obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the standard solution.

System performance: Dissolve 10 mg each of Alminoprofen and butyl parahydroxybenzoate in 100 mL of

methanol. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, alminoprofen and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Alminoprofen, 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
= 21.93 mg of $C_{13}H_{17}NO_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Alminoprofen Tablets

アルミノプロフェン錠

Alminoprofen Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of alminoprofen ($C_{13}H_{17}NO_2$: 219.28).

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

Identification Take an amount of powdered Alminoprofen Tablets, equivalent to 30 mg of Alminoprofen according to the labeled amount, add ethanol (99.5) to make 100 mL, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 253 nm and 257 nm, and between 298 nm and 302 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Powder 10 tablets of Alminoprofen Tablets, weigh a portion of the powder equivalent to 50 mg of Alminoprofen according to the labeled amount, add 50 mL of the mobile phase, shake for 15 minutes, add the mobile phase to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the conditions described in the Purity (3) under Alminoprofen. Determine each peak area of each solution by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/2 times the peak area of alminoprofen from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than 2 times the peak area of alminoprofen 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 Alminoprofen Tablets add 5 mL of water, shake until the tablet is disintegrated, add 50 mL of ethanol (99.5), shake for 20 minutes, then add ethanol (99.5) to make exactly 100 mL, and centrifuge. Pipet 3 mL of the supernatant liquid, add ethanol (99.5) to make exactly 50 mL. Pipet V mL of this solution, add ethanol (99.5) to make exactly V' mL so that each mL contains about 6 μg of alminoprofen ($C_{13}H_{17}NO_2$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of alminoprofen ($C_{13}H_{17}NO_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/3)$

W_S: Amount (mg) of alminoprofen for assay

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 Alminoprofen Tablets is not less than 80%.

Start the test with 1 tablet of Alminoprofen Tablets, withdraw not less than 20 mL of the medium at 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 0.05 mol/L sodium hydroxide TS to make exactly V' mL so that each mL contains about 8.9 μg of alminoprofen ($C_{13}H_{17}NO_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the dessicant, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 245 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of alminoprofen ($C_{13}H_{17}NO_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 27$

W_S: Amount (mg) of alminoprofen for assay
C: Labeled amount (mg) of alminoprofen ($C_{13}H_{17}NO_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Alminoprofen Tablets, and powder. Weigh accurately an amount equivalent to about 60 mg of alminoprofen ($C_{13}H_{17}NO_2$), add ethanol (99.5) and shake well, add ethanol (99.5) to make exactly 200 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add ethanol (99.5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the dessicant, dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at the wavelength of maximum absorption at about 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of alminoprofen } (C_{13}H_{17}NO_2) = W_S \times (A_T/A_S) \times 2$$

W_S: Amount (mg) of alminoprofen for assay

Containers and storage Containers—Well-closed containers.

Add the following:

Alprostadil Injection

アルプロスタジル注射液

Alprostadil Injection is an emulsion-type injection. It contains not less than 80.0% and not more than 125.0% of the labeled amount of alprostadil ($C_{20}H_{34}O_5$: 354.48).

Method of preparation Prepare as directed under Injections, with Alprostadil.

Description Alprostadil Injection occurs as a white emulsion and is slightly viscous. It has a distinctive odor.

Identification To a quantity of Alprostadil Injection, corresponding to 10 μg of Alprostadil according to the labeled amount, add 2 mL of acetonitrile, shake well, and centrifuge. To 3.5 mL of the supernatant liquid add 7 mL of diluted phosphoric acid (1 in 1000), and then run this solution on a column (prepared by filling a 10 mm inside diameter, 9 mm long chromatography tube with 0.4 g of 70 μm octadecylsilanized silica gel for pretreatment) prewashed with 10 mL of methanol and then 10 mL of water. Wash the column with 10 mL of water and then 20 mL of petroleum ether, followed by elution with 2.5 mL of a mixture of methanol and water (4:1). Remove the solvent from the effluent under reduced pressure, dissolve the residue in 100 μL of ethyl acetate, and use this solution as the sample solution. Separately, dissolve 1 mg of Alprostadil Reference Standard in 10 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 the entire volume of the sample solution and 100 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Then, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and acetic acid (100) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (99.5) (1 in 10) on the plate, and heat at 100°C for 5 minutes: the color of the spot obtained from the standard solution and the spot corresponding to that location obtained from the sample solution is dark blue.

pH Being specified separately.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 4.0 mL of Alprostadil Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Prostaglandin A_1—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 10 mg of prostaglandin A_1, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2.5 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine the ratios, Q_T and Q_S, of the peak area of prostaglandin A_1 to that of the internal standard, and calculate the amount of prostaglandin A_1 converted to alprostadil using the following equation: not more than 3.0 μg per a volume, equivalent to 5 μg of alprostadil ($C_{20}H_{34}O_5$).

$$\text{Amount (μg) of prostaglandin } A_1 \text{ } (C_{20}H_{32}O_4), \text{ converted to alprostadil} = W_S \times (Q_T/Q_S) \times (1/2) \times 1.054$$

W_S: Amount (mg) of prostaglandin A_1

Internal standard solution—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add the mobile phase to make 100 mL.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of prostaglandin A_1 obtained with 40 μL of this solution is equivalent to 14 to 26% of that with 40 μL of the standard solution.

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

(3) Peroxide—Pipet 4 mL of Alprostadil Injection, place in a glass-stoppered flask, add 15 mL of a mixture of acetic acid (100) and isooctane (3:2), previously having undergone a 30 minute nitrogen substitution, and dissolve with gentle shaking. To this solution add 0.5 mL of saturat-

ed potassium iodide TS, replace the inside of the vessel with nitrogen, and shake for exactly 5 minutes. Then, add 0.5 mL of starch TS, shake vigorously, add 15 mL of water, and shake vigorously. Under a stream of nitrogen, titrate ⟨*2.50*⟩ with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, perform a blank determination using 4 mL of water, and make any necessary correction. Calculate the amount of peroxides using the following equation: not more than 0.5 meq/L.

$$\text{Amount (meq/L) of peroxides} = V \times 2.5$$

V: Amount (mL) of 0.01 mol/L sodium thiosulfate VS consumed

(4) Free fatty acids—Pipet 3 mL of Alprostadil Injection, add exactly 15 mL of a mixture of 2-propanol, heptane and 0.5 mol/L sulfuric acid TS (40:10:1), and shake for 1 minute. After leaving for 10 minutes, add exactly 9 mL of heptane and exactly 9 mL of water, shake the test tube by inverting 10 times, leave for 15 minutes, and pipet 9 mL of the supernatant liquid. To this solution, add 3 mL of a solution prepared by combining 1 volume of Nile blue solution (1 in 5000) washed 5 times with heptane and 9 volumes of ethanol (99.5), and use this solution as the sample solution. Titrate ⟨*2.50*⟩ this solution with 0.02 mol/L sodium hydroxide VS under a stream of nitrogen. Separately, dissolve 5.65 g of oleic acid in heptane to make exactly 200 mL, and use this solution as the standard solution. Pipet 25 mL of the standard solution, add 2 drops of phenolphthalein TS, titrate ⟨*2.50*⟩ with 0.1 mol/L potassium hydroxide-ethanol VS until a light red color develops, and determine the correction factor f. Pipet 30 mL of the standard solution and add heptane to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 15 mL of a mixture of 2-propanol, heptane and 0.5 mol/L sulfuric acid TS (40:10:1), and shake for 1 minute. After leaving for 10 minutes, add exactly 6 mL of heptane and exactly 12 mL of water, shake the test tube by inverting 10 times, and then titrate ⟨*2.50*⟩ in the same manner as for the sample solution. Determine the volume (mL), V_T and V_S, of 0.02 mol/L sodium hydroxide VS consumed by the sample and standard solutions: the amount of free fatty acid is not more than 12.0 meq/L.

$$\text{Amount (meq/L) of free fatty acids} = (V_T/V_S) \times f \times 15$$

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: no easily detectable foreign matter is observed.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filter method: it meets the requirement. However, use the sample solution consisting of equal volume of Alprostadil Injection and a solution prepared by adding water to 0.1 g of polysorbate 80 to make 100 mL.

Particle diameter Being specified separately.

Assay Measure exactly a volume of Alprostadil Injection corresponding to 5 μg of alprostadil ($C_{20}H_{34}O_5$), add exactly 1 mL of the internal standard solution, shake, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Alprostadil Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in ethanol (99.5) to make exactly 50 mL, and use this solution as standard stock solution. Pipet 2.5 mL of the standard stock solution, add the mobile phase to make exactly 50 mL, pipet 1 mL, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions using an apparatus equipped with an automatic pretreatment device (using a postcolumn reaction), and calculate the ratios, Q_T and Q_S, of the peak area of alprostadil to that of the internal standard.

$$\text{Amount (μg) of alprostadil } (C_{20}H_{34}O_5) = W_S \times (Q_T/Q_S) \times (1/2)$$

W_S: Amount (mg) of Alprostadil Reference Standard

Internal standard solution—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add the mobile phase to make 100 mL.

Operating conditions—

Equipment: Liquid chromatograph consisting of 2 pumps for pumping the mobile phase and the reaction reagent, an automatic pretreatment device, column, reaction coil, detector, and recording apparatus. Use a reaction coil that is maintained at a constant temperature.

Detector: An ultraviolet absorption photometer (wavelength: 278 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 60°C.

Reaction coil: Polytetrafluoroethylene tube 0.5 mm in inside diameter and 10 m in length.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL and adjust the pH to 6.3 by adding a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL. To 1 volume of this solution add 9 volumes of water. To 3 volumes of this solution add 1 volume of acetonitrile for liquid chromatography.

Reaction reagent: Potassium hydroxide TS.

Reaction temperature: A constant temperature of about 60°C.

Mobile phase flow rate: Adjust the flow rate so that the retention time of alprostadil is about 7 minutes.

Reaction reagent flow rate: 0.5 mL per minute.

Automatic pretreatment device: Composed of a pretreatment column, pump for pumping pretreatment column wash solution, and routing valve for 2 high pressure flow paths.

Pretreatment column: A stainless steel column 4 mm in inside diameter and 2.5 cm in length, packed with octadecyl-

silanized silica gel for liquid chromatography (5 μm in particle diameter).

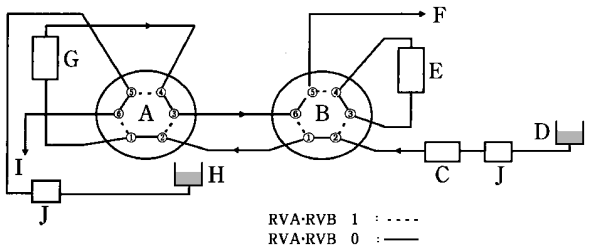
Pretreatment column wash solution: Ethanol (99.5).

Flow rate of wash solution: A constant flow rate of about 2.0 mL per minute.

Flow path operating conditions: Change the flow path operating conditions at the times shown in the table below using the valves shown in the figure.

	Time of switchover (minutes)				
Valve	0	9.0	9.1	*1)	*2)
RVA	0	0	1	0	0
RVB	0	1	1	1	0

*1) After the internal standard has completely eluted
*2) 0.1 minutes after *1)



A: RVA valve
B: RVB valve
C: Sample injector
D: Mobile phase
E: Column for pressure correction
F: Column
G: Pretreatment column
H: Wash solution
I: Drain
J: Pump

Figure Components of automatic pretreatment system

System suitability—

System performance: Dissolve 10 mg of prostaglandin A_1, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, in ethanol (99.5) to make 100 mL. To 2.5 mL of this solution add 2.5 mL of the standard stock solution, and add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of the internal standard solution, shake, and perform the test under the above conditions with 40 μL of the solution. Alprostadil, prostaglandin A_1 and the internal standard are eluted in this order, and the resolution between the peaks of alprostadil and prostaglandin A_1 is not less than 10, and that between prostaglandin A_1 and the internal standard is not less than 2.0.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, not exceeding 5°C, avoiding freezing.

Add the following:

Amikacin Sulfate Injection

アミカシン硫酸塩注射液

Amikacin Sulfate Injection is an aqueous injection. It contains not less than 90.0% and not more than 115.0% of the labeled amount of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60).

Method of preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate Injection occurs as a colorless or pale yellow clear liquid.

Identification To a volume of Amikacin Sulfate Injection, equivalent to 0.1 g (potency) of Amikacin Sulfate according to the labeled amount, add water to make 4 mL, and use this solution as the sample solution. Separately, dissolve 25 mg (potency) of Amikacin Sulfate Reference Standard in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identifiction (2) under Amikacin Sulfate.

Osmotic pressure ratio Being specified separately.

pH <*2.54*> 6.0 – 7.5

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 Take exactly a volume of Amikacin Sulfate Injection, equivalent to about 0.1 g (potency) of Amikacin Sulfate, and add water to make exactly 100 mL. Separately, weigh accurately an amount of Amikacin Sulfate Reference Standard, equivalent to about 50 mg (potency), and add water to make exactly 50 mL. Take exactly 200 μL each of these solutions into stoppered test tubes, then proceed as directed in the Assay under Amikacin Sulfate.

Amount [mg (potency)] of amikacin ($C_{22}H_{43}N_5O_{13}$)
$= W_S \times (H_T/H_S) \times 2$

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

Container and storage Containers—Hermetic containers.

Aminophylline Injection

アミノフィリン注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.6 EU/mg.

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.

Amitriptyline Hydrochloride Tablets

アミトリプチリン塩酸塩錠

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 Amitriptyline Hydrochloride Tablets add 50 mL of diluted methanol (1 in 2), shake to disintegrate the tablet, then add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V' mL so that each mL contains about 10 μg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

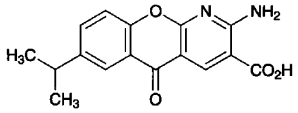
Amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$) $= W_S \times (A_T/A_S) \times (V'/V) \times (1/20)$

W_S: Amount (mg) of Amitriptyline Hydrochloride Reference Standard

Add the following:

Amlexanox

アンレキサノクス



$C_{16}H_{14}N_2O_4$: 298.29
2-Amino-7-(1-methylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridine-3-carboxylic acid
[*68302-57-8*]

Amlexanox, when dried, contains not less than 98.0 % and not more than 102.0% of $C_{16}H_{14}N_2O_4$.

Description Amlexanox occurs as white to yellowish white crystals or crystalline powder.

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

It dissolves in diluted sodium hydroxide TS (1 in 3).

Identification **(1)** Determine the absorption spectrum of a solution of Amlexanox in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlexanox 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 Amlexanox 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 Amlexanox Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Amlexanox in 20 mL of water and 10 mL of sodium hydroxide TS, add 15 mL of dilute nitric acid and water to make 50 mL, centrifuge, and then filter the supernatant liquid. To 25 mL of this filtrate add water to make 50 mL. Perform the test using this solution as the test solution. The control solution consists of 5 mL of sodium hydroxide TS, 7.5 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS, and water added to make 50 mL (not more than 0.021%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Amlexanox 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—(i) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 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 these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox from the standard solution.

Operating conditions—

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

Time span of measurement: Until completion of the elution of amlexanox beginning after the solvent peak.

System suitability—

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

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

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

(ii) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 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, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: To 15 mL of a solution of benzophenone in the mobile phase (3 in 1,000,000) add the mobile phase to make 20 mL. Adjust the flow rate so that the retention time of benzophenone is about 6.5 minutes when perform the test with 10 μL of this solution under the conditions described above.

Time span of measurement: About 3 times as long as the retention time of benzophenone, beginning after the peak of amlexanox.

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

System performance: Pipet 1 mL of the sample solution, and add the mobile phase to make 100 mL. To 5 mL of this solution add 15 mL of the solution of benzophenone in the mobile phase (3 in 1,000,000). When perform the test with 10 μL of this solution according to the above conditions, amlexanox 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlexanox is not more than 2.0%.

(iii) The total amount of related substances, when calculated according to the following formula, is not more than 0.5%.

$$\text{Total amount (\%) of related substances} = \{(A_{T1}/A_{S1}) + (A_{T2}/A_{S2})\} \times (1/10)$$

A_{T1}: Total area of the peaks other than amlexanox from the sample solution obtained in (i)

A_{T2}: Total area of the peaks other than amlexanox from the sample solution obtained in (ii)

A_{S1}: Peak area of amlexanox from the standard solution obtained in (i)

A_{S2}: Peak area of amlexanox from the standard solution obtained in (ii)

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 30 mg each of Amlexanox and Amlexanox Reference Standard, both dried, and dissolve them separately in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, and add exactly 15 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and 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 amlexanox to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{16}H_{14}N_2O_4 = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Amlexanox Reference Standard

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 4000).

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 17.9 g of disodium hydrogen

phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 760 mL of this solution add 240 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution according to the above conditions, amlexanox 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 conditions, the relative standard deviation of the ratio of the peak area of amlexanox to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Amlexanox Tablets

アンレキサノクス錠

Amlexanox Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amlexanox ($C_{16}H_{14}N_2O_4$: 298.29).

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

Identification (1) Take an amount of powdered Amlexanox Tablets, equivalent to 10 mg of Amlexanox according to the labeled amount, add 100 mL of ethanol (99.5), shake vigorously, and filter. Pipet 1 mL of the filtrate, add 25 mL of ethanol (99.5), and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*: it exhibits absorption maxima between 240 nm and 244 nm, between 285 nm and 289 nm, and between 341 nm and 352 nm.

(2) Observe the sample solution obtained in (1) under ultraviolet light (main wavelength: 365 nm): the solution shows a bluish-white fluorescence.

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 Amlexanox Tablets, add exactly 0.6 mL of the internal standard solution per 1 mg of amlexanox ($C_{16}H_{14}N_2O_4$), add the mobile phase to make exactly V mL so there is about 167 μg of amlexanox ($C_{16}H_{14}N_2O_4$) per 1 mL, disintegrate the tablet, and then shake vigorously for 5 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Amlexanox Reference Standard, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 25 mL of this solution, 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 Amlexanox.

$$\text{Amount (mg) of amlexanox } (C_{16}H_{14}N_2O_4) = W_S \times (Q_T/Q_S) \times (V/200)$$

W_S: Amount (mg) of Amlexanox Reference Standard

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 500).

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 Amlexanox Tablets is not less than 80%.

Start the test with 1 tablet of Amlexanox 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 5.6 μg of amlexanox ($C_{16}H_{14}N_2O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Amlexanox Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 2 mL of dilute sodium hydroxide TS, add the dissolution medium to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 350 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry *⟨2.24⟩*.

$$\text{Dissolution rate (\%) with respect to the labeled amount of amlexanox } (C_{16}H_{14}N_2O_4) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S: Amount (mg) of Amlexanox Reference Standard

C: Labeled amount (mg) of amlexanox ($C_{16}H_{14}N_2O_4$) in 1 tablet

Assay Weigh accurately not less than 20 Amlexanox Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of amlexanox ($C_{16}H_{14}N_2O_4$), add exactly 10 mL of the internal standard solution, add 80 mL of the mobile phase, shake vigorously for 5 minutes, and then add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Amlexanox Reference Standard, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solu-

tion. Then, proceed as directed in the Assay under Amlexanox.

$$\text{Amount (mg) of amlexanox } (C_{16}H_{14}N_2O_4) = W_S \times (Q_T/Q_S) \times (1/2)$$

W_S: Amount (mg) of Amlexanox Reference Standard

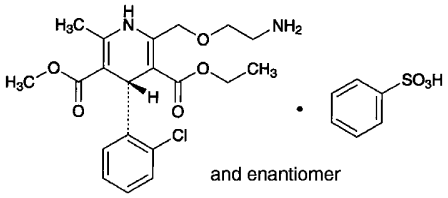
Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.

Add the following:

Amlodipine Besilate

アムロジピンベシル酸塩



$C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05
3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate monobenzenesulfonate [*111470-99-6*]

Amlodipine Besilate contains not less than 98.0% and not more than 102.0% of $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$, calculated on the anhydrous basis.

Description Amlodipine Besilate occurs as a white to yellowish white crystalline powder.

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

A solution of Amlodipine Besilate in methanol (1 in 100) shows no optical rotation.

Melting point: about 198°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Amlodipine Besilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlodipine Besilate 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 Amlodipine Besilate 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 Amlodipine Besilate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 30 mg of Amlodipine Besilate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and add barium chloride TS: a white precipitate is formed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Amlodipine Besilate according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Related substances—Dissolve 0.10 g of Amlodipine Besilate in 50 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 3 mL of this solution, add the mixture of water and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of 0.90 with respect to amlodipine, obtained from the sample solution is not larger than the peak area of amlodipine from the standard solution, and the area of the peak other than amlodipine, other than benzenesulfonic acid having the relative retention time of about 0.15 with respect to amlodipine, and other than the peak mentioned above is not larger than 1/3 times the peak area of amlodipine from the standard solution. Furthermore, total peak area for peaks other than amlodipine and benzenesulfonic acid of the sample solution is not larger than 2.7 times the peak area of amlodipine from the standard solution.

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 (3 μm in particle diameter).

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

Mobile phase A: A mixture of water and trifluoroacetic acid (5000:1).

Mobile phase B: A mixture of acetonitrile and trifluoroacetic acid (5000:1).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	80→20	20→80
30 – 45	20	80

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of amlodipine, 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 acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of amlodipine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 70,000 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 amlodipine is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water ⟨*2.48*⟩ Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 35 mg each of Amlodipine Besilate and Amlodipine Besilate Reference Standard (separately determine the water ⟨*2.48*⟩ using the same manner as Amlodipine Besilate), dissolve them separately in the mobile phase to make exactly 250 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL 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.

$$\text{Amount (mg) of } C_{20}H_{25}ClN_2O_5.C_6H_6O_3S = W_S \times (Q_T/Q_S)$$

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 20,000).

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 25°C.

Mobile phase: A mixture of methanol and a solution of potassium dihydrogen phosphate (41 in 10,000) (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 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 amlodipine to that of the internal standard is not more than 1.0%.

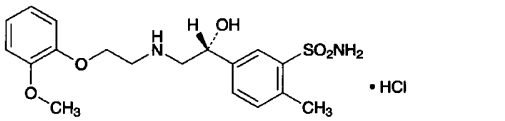
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Amosulalol Hydrochloride

アモスラロール塩酸塩



and enantiomer

$C_{18}H_{24}N_2O_5S.HCl$: 416.92

5-((1*RS*)-1-Hydroxy-2-{[2-(2-methoxyphenoxy)ethyl]amino}ethyl)-2-methylbenzenesulfonamide monohydrochloride

[*70958-86-0*]

Amosulalol Hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{18}H_{24}N_2O_5S.HCl$, calculated on the anhydrous basis.

Description Amosulalol Hydrochloride occurs as white crystals or a white crystalline powder. It has a bitter taste.

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

It is hygroscopic.

A solution of Amosulalol Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Amosulalol Hydrochloride (1 in 20,000) 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 Amosulalol 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 Amosulalol Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Melting point <2.60> 158 – 162°C

Purity (1) Heavy metals <1.07>—Place 1.0 g of Amosulalol Hydrochloride in a porcelain crucible, add 1.5 mL of sulfuric acid, cover loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid, heat carefully until white fumes no longer are evolved, and then heat intensely to 500 – 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed according to Method 2, and perform the test. The control solution, processed in the same manner as the test solution using the same amounts of reagents, is prepared by combining 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Amosulalol Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 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, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than amosulalol obtained from the sample solution is not larger than 2/5 times the peak area of amosulalol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

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

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a solution prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.

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

Time span of measurement: About 2 times as long as the retention time of amosulalol, 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 amosulalol obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amosulalol are not less than 4000 and not more than 1.7, 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 amosulalol is not more than 1.0%.

Water <2.48> Not more than 4.0% (1 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.6 g of Amosulalol Hydrochloride, dissolve in 3 mL of formic acid, add 80 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and titrate <2.50> within 5 minutes with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination using the same procedure, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.69 mg of $C_{18}H_{24}N_2O_5S.HCl$

Containers and storage Containers—Tight containers.

Add the following:

Amosulalol Hydrochloride Tablets

アモスラロール塩酸塩錠

Amosulalol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$: 416.92).

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

Identification To a quantity of powdered Amosulalol Hydrochloride Tablets, equivalent to 50 mg of Amosulalol Hydrochloride according to the labeled amount, add 25 mL of 0.1 mol/L hydrochloric acid TS, shake well, and then centrifuge. To 2.5 mL of the supernatant liquid 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 270 nm and 274 nm, and a shoulder between 275 nm and 281 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 1 tablet of Amosulalol Hydrochloride Tablets, disintegrate by adding 2 mL of 0.1 mol/L hydrochloric acid TS, add 15 mL of methanol, and shake well. Add methanol to make exactly *V* mL so that each mL contains about 0.4 mg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of amosulalol hydrochloride for assay (separately determine the water <2.48> in the same manner as Amosulalol Hydrochloride),

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 the mobile phase to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = W_S \times (Q_T/Q_S) \times (V/50)$$

W_S: Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

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 Amosulalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Amosulalol 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 5.5 μg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of amosulalol hydrochloride for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Amosulalol Hydrochloride), and dissolve in water to make exactly 200 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 amosulalol peak areas, A_T and A_S, of both solutions.

$$\text{Dissolution rate (\%) with respect to the labeled amount of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (45/2)$$

W_S: Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

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

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a soluion prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of amosulalol is about 5 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 amosulalol are not less than 4000 and not more than 1.7, 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 amosulalol is not more than 1.0%.

Assay Take 10 Amosulalol Hydrochloride Tablets, add 20 mL of 0.1 mol/L hydrochloric acid TS, and shake well to disintegrate. Add 120 mL of methanol, again shake well, add methanol to make exactly 200 mL, and then centrifuge. Pipet a volume of supernatant liquid corresponding to about 5 mg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), 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 amosulalol hydrochloride for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Amosulalol Hydrochloride), and dissolve in methanol to make exactly 25 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 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 amosulalol to that of the internal standard.

$$\text{Amount (mg) of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = W_S \times (Q_T/Q_S) \times (1/5)$$

W_S: Amount (mg) of amosulalol hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 diluted acetic acid (100) (1 in 25), acetonitrile and a solution of ammonium acetate (1 in 250) (5:3:2).

Flow rate: Adjust the flow rate so that the retention time

of amosulalol is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, amosulalol and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

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

Containers and storage Containers—Tight containers.

Add the following:

Ampicillin Sodium for Injection

注射用アンピシリンナトリウム

Ampicillin Sodium 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 amount of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Method of preparation Prepare as directed under Injections, with Ampicillin Sodium.

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

Identification Proceed as directed in the Identification (1) under Ampicillin Sodium.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Ampicillin Sodium for Injection, equivalent to 1.0 g (potency) of Ampicillin Sodium according to the labeled amount, in 10 mL of water is 8.0 to 10.0.

Purity Clarity and color of solution—Dissolve an amount of Ampicillin Sodium for Injection, equivalent to 0.25 g (potency) of Ampicillin Sodium according to the labeled amount, in 0.75 mL of water: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 400 nm is not more than 0.40.

Water ⟨*2.48*⟩ Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.075 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 of the contents of not less than 10 containers of Ampicillin Sodium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 50 mg (potency) of Ampicillin Sodium, add exactly 5 mL of the internal standard solution and dissolve. Then add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution and dissolve. Then 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 ampicillin to that of the internal standard.

Amount [mg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
$= W_S \times (Q_T/Q_S)$

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

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

Operating conditions—

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

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

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

Mobile phase: Dissolve 5.94 mg of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitril, add phosphoric acid to adjust the pH to 5.0, then add water to make exactly 1000 mL.

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 26.

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 ampicillin to that of the internal standard is not more than1.0%.

Containers and storage Containers—Hermetic containers.

L-Arginine Hydrochloride Injection

L-アルギニン塩酸塩注射液

Delete the Pyrogen and add the following next to pH:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mL.

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.

Ascorbic Acid Injection

アスコルビン酸注射液

Add the following next to pH:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.15 EU/mg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

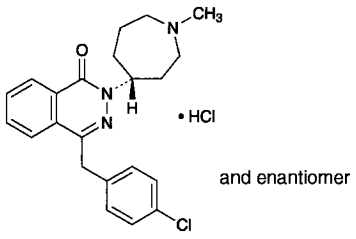
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Azelastine Hydrochloride

アゼラスチン塩酸塩



$C_{22}H_{24}ClN_3O.HCl$: 418.36

4-[(4-Chlorophenyl)methyl]-2-[(4*RS*)-(1-methylazepan-4-yl)]phthalazin-1(2*H*)-one monohydrochloride [*79307-93-0*]

Azelastine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{22}H_{24}ClN_3O.HCl$.

Description Azelastine Hydrochloride occurs as a white crystalline powder.

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

Melting point: about 225°C (with decomposition).

A solution of Azelastine Hydrochloride (1 in 200) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Azelastine Hydrochloride (3 in 100,000) 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 Azelastine 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) To 10 mL of a saturated solution of Azelastine Hydrochloride add 1 mL of dilute nitric acid, and filter to separate formed crystals: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Azelastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Azelastine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Azelastine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than azelastine obtained from the sample solution is not larger than 1/10 times the peak area of azelastine from the standard solution, and the total area of the peaks other than the peak of azelastine from the sample solution is not larger than 1/2 times the peak area of azelastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

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

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

Mobile phase: A mixture of water, acetonitrile and perchloric acid (660:340:1).

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

Time span of measurement: About 2 times as long as the retention time of azelastine, 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 50 mL. Confirm that the peak area of azelastine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine is 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 azelastine is not more than 1.0%.

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 previously dried Azelastine Hydrochloride, dissolve in 5 mL of formic acid, add 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
= 41.84 mg of $C_{22}H_{24}ClN_3O.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Aztreonam for Injection

注射用アズトレオナム

Aztreonam 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 amount of aztreonam ($C_{13}H_{17}N_5O_8S_2$: 435.43).

Method of preparation Prepare as directed under Injections, with Aztreonam.

Description Aztreonam for Injection is white to yellowish white masses or powder.

Identification **(1)** Dissolve an amount of Aztreonam for Injection, equivalent to 6 mg (potency) of Aztreonam according to the labeled amount, in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and mix: a red-brown color develops.

(2) Dissolve an amount of Aztreonam for Injection, equivalent to 3 mg (potency) of Aztreonam according to the labeled amount, in 100 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 289 nm and 293 nm.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of Aztreonam according to the labeled amount, in 10 mL of water is 4.5 to 7.0.

Purity Clarity and color of solution—Dissolve an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of Aztreonam according to the labeled amount, in 10 mL of water: the solution is clear, and its absorbance ⟨*2.24*⟩ at 450 nm is not more than 0.06.

Water ⟨*2.48*⟩ Not more than 2.0% (0.5 g, volumetric titration, direct titration).

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 Take an amount of Aztreonam for Injection, equivalent to about 5 g (potency) of Aztreonam, dissolve the contents with a suitable amount of water, and transfer to a 100-mL volumetric flask. Wash each container with water, combine the washings and the solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aztreonam Reference Standard, equivalent to about 20 mg (potency), dissolve in a suitable amount of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Aztreonam.

Amount [mg (potency)] of aztreonam ($C_{13}H_{17}N_5O_8S_2$)
$= W_S \times (Q_T/Q_S) \times 250$

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

Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250).

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

Baclofen Tablets

バクロフェン錠

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 Baclofen Tablets add 5 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into small particles with the aid of ultrasonic waves, then shake for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of baclofen ($C_{10}H_{12}ClNO_2$). Centrifuge, pipet 5 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Baclofen Reference Standard (separately determine the water ⟨*2.48*⟩ in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the standard solution. To exactly 2 mL each of the sample solution and standard solution add 4 mL of ninhydrin-tin (II) chloride TS, mix, heat on a water bath for 20 minutes, then immediately shake vigorously for 2 minutes. After cooling, add a mixture of water and 1-propanol (1:1) to make them exactly 25 mL, and determine the absorbances, A_T and A_S, of them at 570 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution obtained with 2 mL of water by the same procedure as above as the blank.

$$\text{Amount (mg) of baclofen } (C_{10}H_{12}ClNO_2) = W_S \times (A_T/A_S) \times (V/50)$$

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

Add the following:

Benzylpenicillin Potassium for Injection

注射用ベンジルペニシリンカリウム

Benzylpenicillin Potassium 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 amount of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$: 372.48).

Method of preparation Prepare as directed under Injections, with Benzylpenicillin Potassium.

Description Benzylpenicillin Potassium for Injection occurs as white crystals or crystalline powder.

Identification Proceed as directed in the Identification (2) under Benzylpenicillin Potassium.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equivalent to 1.0×10^5 Units of Benzylpenicillin Potassium according to the labeled amount, in 10 mL of water is 5.0 to 7.5.

Purity Clarity and color of solution—A solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equivalent to 1.0×10^6 Units of Benzylpenicillin Potassium according to the labeled amount, in 10 mL of water is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 400 nm is not more than 0.10.

Loss on drying ⟨*2.41*⟩ Not more than 1.2% (3 g, in vacuum, below 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.25×10^{-4} EU/Unit.

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 of the contents of not less than 10 containers of Benzylpenicillin Potassium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 6×10^4 Units of Benzylpenicillin Potassium, dissolve in water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium Reference Standard, equivalent to about 6×10^4 Units, dissolve in water 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 the peak area of benzylpenicillin, A_T and A_S, from each solution.

$$\text{Amount (unit) of Benzylpenicillin Potassium } (C_{16}H_{17}KN_2O_4S) = W_S \times (A_T/A_S)$$

W_S: Amount (unit) of Benzylpenicillin Potassium Reference Standard

Operating conditions—

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

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

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

Mobile phase: To a mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitril (19:6), add phosphoric acid to adjust the pH of this solution to 8.0.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 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 benzylpenicillin are not less than 6000 and not more than 2.0, 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 benzylpenicillin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Betahistine Mesilate

ベタヒスチンメシル酸塩

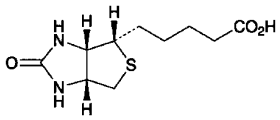
Change the Identification (3) to read:

Identification (3) A 30 mg portion of Betahistine Mesilate responds to the Qualitative Tests <*1.09*> (2) for mesilate.

Add the following:

Biotin

ビオチン



$C_{10}H_{16}N_2O_3S$: 244.31

5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid [*58-85-5*]

Biotin, when dried, contains not less than 98.5% and not more than 101.0% of $C_{10}H_{16}N_2O_3S$.

Description Biotin occurs as white crystals or a white crystalline powder.

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

It dissolves in dilute sodium hydroxide TS.

Melting point: about 231°C (with decomposition).

Identification Determine the infrared absorption spectrum of Biotin 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.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +89 – +93° (after drying, 0.4 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Biotin in 10 mL of 0.5 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Biotin 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*>—Place 0.7 g of Biotin in a Kjeldahl flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and carefully heat until white fumes are evolved. After cooling, add 2 mL of nitric acid twice, heat, add 2 mL of hydrogen peroxide (30) several times, and heat until the color of the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate solution, and heat to concentrate until white fumes are evolved again. After cooling, add water to make 5 mL, and perform the test using this solution as the test solution (not more than 2.8 ppm).

(4) Related substances—Dissolve 0.10 g of Biotin in 10 mL of diluted ammonia solution (28) (7 in 100), and use this solution as the sample solution. Pipet 1 mL of this solution, and add diluted ammonia solution (28) (7 in 100) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted ammonia solution (28) (7 in 100) 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 1-butanol, water, and acetic acid (100) (5:2:1) to a distance of about 10 cm, air-dry the plate, and then dry for 30 minutes at 105°C. Spray the plate evenly with a mixture of a solution of 4-dimethylaminocinnamaldehyde in ethanol (99.5) (1 in 500) and a solution of sulfuric acid in ethanol (99.5) (1 in 50) (1:1): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

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

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

Assay Weigh accurately about 0.25 g of Biotin, previously dried, dissolve by adding exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and titrate <*2.50*> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $C_{10}H_{16}N_2O_3S$

Containers and storage Containers—Tight containers.

Bisacodyl Suppositories

ビサコジル坐剤

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 suppository of Bisacodyl Suppositories add tetrahydrofuran to make a solution containing about 0.2 mg of bisacodyl ($C_{22}H_{19}NO_4$) in each mL, warm to 40°C, and shake to dissolve. After cooling, add tetrahydrofuran to make exactly V mL so that each mL contains about 10 μg of bisacodyl ($C_{22}H_{19}NO_4$). Pipet 5 mL of this solution, and proceed as directed in the Assay.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = W_S \times (Q_T/Q_S) \times (V/50)$$

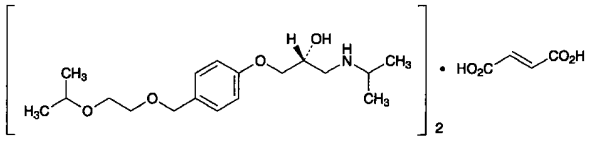
W_S: Amount (mg) of Bisacodyl Reference Standard

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

Add the following:

Bisoprolol Fumarate

ビソプロロールフマル酸塩



and enantiomer

$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$: 766.96

(2*RS*)-1-(4-{[2-(1-Methylethoxy)ethoxy]methyl}phenoxy)-3-[(1-methylethyl)amino]propan-2-ol hemifumarate [*104344-23-2*]

Bisoprolol Fumarate, when dried, contains not less than 98.5% and not more than 101.0% of $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$.

Description Bisoprolol Fumarate occurs as white crystals or a white crystalline powder.

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

A solution of Bisoprolol Fumarate (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Bisoprolol Fumarate in methanol (1 in 10,000) 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 Bisoprolol Fumarate 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.

Melting point ⟨*2.60*⟩ 101 – 105°C

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Bisoprolol Fumarate 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 50 mg of Bisoprolol Fumarate in 100 mL of a mixture of water and acetonitrile (4:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add the mixture of water and acetonitrile (4:1) 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 peaks other than bisoprolol obtained from the sample solution is not larger than 1/2 times the peak area of bisoprolol from the standard solution. Furthermore, the total of the areas of all peaks other than bisoprolol from the sample solution is not larger than the peak area of bisoprolol 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 octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

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

Time span of measurement: About 2 times as long as the retention time of bisoprolol, beginning after the fumaric acid peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and acetonitrile (4:1) to make exactly 20 mL. Confirm that the peak area of bisoprolol obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol 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 bisoprolol is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Bisoprolol Fumarate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). The endpoint of titration is when the purple color of the solution turns blue and then blue-green. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.35 mg of $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Add the following:

Bisoprolol Fumarate Tablets

ビソプロロールフマル酸塩錠

Bisoprolol Fumarate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4$: 766.96].

Method of preparation Prepare as directed under Tablets, with Bisoprolol Fumarate.

Identification To a quantity of powdered Bisoprolol Fumarate Tablets, equivalent to 10 mg of Bisoprolol Fumarate according to the labeled amount, add 60 mL of methanol, shake vigorously for 10 minutes, add methanol to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum 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 1 tablet of Bisoprolol Fumarate Tablets, disintegrate by adding 8 mL of water, and add water to make exactly 10 mL, and then 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, add water to make exactly V' mL so that each mL contains about 0.1 mg of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$, and use as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried under reduced pressure at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, dissolve in water to make exactly 200 mL, and use as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 271.5 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/20)$

W_S: Amount (mg) of bisoprolol fumarate for assay

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 30 minutes of Bisoprolol Fumarate Tablets is not less than 85%.

Start the test with 1 tablet of Bisoprolol Fumarate 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 bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$ according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours using phosphorus (V) oxide as a dessicant, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 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 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 bisoprolol peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S: Amount (mg) of bisoprolol fumarate for assay
C: Labeled amount (mg) of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$ in 1 tablet

Operating conditions—

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

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 750 mL of this solution add 250 mL of acetonitrile.

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 bisoprolol 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 bisoprolol is not more than 2.0%.

Assay Weigh accurately not less than 20 Bisoprolol Fumarate Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$], add exactly 70 mL of a mixture of water and acetonitrile (3:1) and exactly 10 mL of the internal standard solution, shake vigorously for 10 minutes, and add the mixture of water and acetonitrile (3:1) to make 100 mL. Filter this solution through a membrane filter with a 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 about 20 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours using phosphorus (V) oxide as the desiccant, add exactly 10 mL of the internal standard solution, dissolve in the mixture of water and acetonitrile (3:1) 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 bisoprolol to that of the internal standard.

Amount (mg) of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$]
$= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of bisoprolol fumarate for assay

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mixture of water and acetonitrile (3:1) (1 in 250).

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 octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bisoprolol 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, fumaric acid, bisoprolol and the internal standard are eluted in this order with the resolution between the peaks of bisoprolol and the internal standard being not less than 12.

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

Containers and storage Containers—Tight containers.

Add the following:

Bucillamine Tablets

ブシラミン錠

Bucillamine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bucillamine ($C_7H_{13}NO_3S_2$: 223.31).

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

Identification **(1)** To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine according to the labeled amount, add 0.1 g of sodium hydrogen carbonate and 10 mL of water, shake well, filter, and add 1 or 2 drops of ninhydrin TS to the filtrate: it exhibits a red-brown color.

(2) To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine according to the labeled amount, add 25 mL of water, shake well, and filter. To 5 mL of the filtrate, add 2 mL of dilute sodium hydroxide TS and 1 or 2 drops of sodium pentacyanonitrosylferrate (III) TS: it exhibits a red-purple color.

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

Store the sample solution and standard solution in a cold place until performing the measurements. Take 1 tablet of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), then add 3 mL of water and 6 mL of methanol per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), and stir well until the tablet completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$)
$= W_S \times (Q_T/Q_S) \times C \times (1/200)$

W_S: Amount (mg) of bucillamine for assay
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).

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 Bucillamine Tablets is not less than 80%.

Store the sample solution and standard solution in a cold place until performing the measurements. Start the test with 1 tablet of Bucillamine 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 an amount of bucillamine

for assay equivalent to the labeled amount of the tablet, previously dried in vacuum at 60°C for 6 hours using phosphorus (V) oxide as a dessicant, and dissolve in methanol to make exactly 10 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 bucillamine peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of bucillamine ($C_7H_{13}NO_3S_2$)
$= W_S \times (A_T/A_S) \times (1/C) \times 90$

W_S: Amount (mg) of bucillamine for assay
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (11:9).

Flow rate: Adjust the flow rate so that the retention time of bucillamine is about 4 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 bucillamine 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 bucillamine is not more than 2.0%.

Assay Store the sample solution and standard solution in a cold place until performing the measurements. Take 10 tablets of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), add 3 mL of water and 6 mL of methanol, and stir well until the tablets completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of bucillamine for assay, previously dried in vacuum for 6 hours at 60°C using phosphorus (V) oxide as a dessicant, add exactly 2 mL of the internal standard solution, and add 6 mL of water and 12 mL of methanol. To 1 mL of this solution add 25 mL of the mobile phase, filter through a membrane filter with a pore size not exceeding 0.45 μm, 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 bucillamine to that of the internal standard.

Amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$)
$= W_S \times (Q_T/Q_S) \times C \times (1/200)$

W_S: Amount (mg) of bucillamine for assay
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 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 diluted phosphoric acid (1 in 1000) and methanol (3:2).

Flow rate: Adjust the flow rate so that the retention time of bucillamine 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, bucillamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

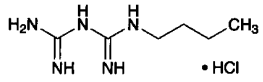
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bucillamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Buformin Hydrochloride

ブホルミン塩酸塩



$C_6H_{15}N_5.HCl$: 193.68
1-Butylbiguanide hydrochloride [*1190-53-0*]

Buformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_6H_{15}N_5.HCl$.

Description Buformin Hydrochloride occurs as a white crystalline powder.

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

Identification (1) To 5 mL of a solution of Buformin Hydrochloride (1 in 2000) add 1 mL of dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate

(III) TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Buformin Hydrochloride (1 in 125,000) 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) Determin the infrared absorption spectrum of Buformin 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.

(4) A solution of Buformin Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chlorides.

Melting point <*2.60*> 175 – 180°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Buformin 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) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Buformin Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Buformin Hydrochloride in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and 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: the area of the peak other than buformin obtained from the sample solution is not larger than 1/5 times the peak area of buformin from the standard solution. Furthermore, the total of the areas of all peaks other than the buformin peak from the sample solution is not larger than 1/2 times the peak area of buformin from the standard solution.

Operating conditions—

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

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

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

Mobile phase: A mixture of a solution of sodium perchlorate monohydrate in diluted phosphoric acid (1 in 1000) (7 in 250) and acetonitrile (7:1).

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

Time span of measurement: About 2 times as long as the retention time of buformin, 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 buformin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buformin 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 buformin is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.15 g of Buformin Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and immediately 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
= 9.684 mg of $C_6H_{15}N_5.HCl$

Containers and storage Containers—Tight containers.

Add the following:

Buformin Hydrochloride Enteric-coated Tablets

ブホルミン塩酸塩腸溶錠

Buformin Hydrochloride Enteric-coated Tablets contain not less than 93.0% and not more than 107.0 % of the labeled amount of buformin hydrochloride ($C_6H_{15}N_5.HCl$: 193.68).

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

Identification To a quantity of powdered Buformin Hydrochloride Enteric-coated Tablets, equivalent to 0.1 g of Buformin Hydrochloride according to the labeled amount, add 10 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of a mixture of hydrogen peroxide TS, sodium pentacyanonitrosylferrate (III) TS and a solution of sodium hydroxide (1 in 10) (2:1:1): the solution exhibits a red to red-purple color.

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 Buformin Hydrochloride Enteric-coated Tablets add 5 mL of a mixture of ethanol (99.5) and acetone

(1:1), disperse the pellicle to smaller using ultrasonic waves, add exactly 10 mL of the internal standard solution per 50 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), and then add diluted acetonitrile (1 in 2) to make $13V/20$ mL. Disintegrate the tablet using ultrasonic waves, then shake for 20 minutes, and add diluted acetonitrile (1 in 2) to make a solution, volume V mL, containing about 0.5 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl) per mL. Centrifuge this solution, pipet 1 mL of the supernatant liquid, and add the mobile phase to make 50 mL. If necessary, filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (Q_T/Q_S) \times (V/50)$

W_S: Amount (mg) of buformin hydrochloride for assay

Internal standard solution—A solution of *p*-acetanisidide in diluted acetonitrile (1 in 2) (1 in 150).

Dissolution *⟨6.10⟩* When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st fluid for dissolution test and 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 120 minutes of Buformin Hydrochloride Enteric-coated Tablets using the 1st fluid is not more than 5%, and that in 90 minutes of Buformin Hydrochloride Enteric-coated Tablets using the 2nd fluid is not less than 80%.

Start the test with 1 tablet of Buformin Hydrochloride Enteric-coated 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 the relevant dissolution medium to make exactly V' mL so that each mL contains about 56 μg of buformin hydrochloride ($C_6H_{15}N_5$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the relevant dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the relevant 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 buformin peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$

W_S: Amount (mg) of buformin hydrochloride for assay
C: Labeled amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) in 1 tablet

Operating conditions—

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

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

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

Mobile phase: A mixture of a solution of sodium perchlorate in diluted phosphoric acid (1 in 1000) (7 in 500) and acetonitrile (7:1).

Flow rate: Adjust the flow rate so that the retention time of buformin 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 buformin 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 buformin is not more than 2.0%.

Assay Add 20 mL of a mixture of ethanol (99.5) and acetone (1:1) to an amount of Buformin Hydrochloride Enteric-coated Tablets equivalent to 0.5 g of buformin hydrochloride ($C_6H_{15}N_5$.HCl), disperse the pellicles to smaller using ultrasonic waves, and then add 100 mL of diluted acetonitrile (1 in 2). Disintegrate the tablets with the aid of ultrasonic waves, shake for 20 minutes, and then 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, and then add diluted acetonitrile (1 in 2) to make 50 mL. Pipet 1 mL of this solution, and add the mobile phase to make 50 mL. If necessary, filter this solution 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 25 mL of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in an adequate amount of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, and then add diluted acetonitrile (1 in 2) to make 50 mL. To 1 mL of this 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 buformin to that of the internal standard.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (Q_T/Q_S) \times 20$

W_S: Amount (mg) of buformin hydrochloride for assay

Internal standard solution—A solution of *p*-acetanisidide in diluted acetonitrile (1 in 2) (1 in 150).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 233 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of a solution of sodium perchlorate (7 in 250) and acetonitrile (7:1).

Flow rate: Adjust the flow rate so that the retention time of buformin 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, buformin 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 buformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Buformin Hydrochloride Tablets

ブホルミン塩酸塩錠

Buformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl: 193.68).

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

Identification To a quantity of powdered Buformin Hydrochloride Tablets, equivalent to 1 g of Buformin Hydrochloride according to the labeled amount, add 100 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS: the solution exhibits a red-brown color.

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 Buformin Hydrochloride Tablets, add water to make exactly 200 mL, and then treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution and centrifuge. Pipet V mL of the supernatant liquid equivalent to about 0.5 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 2 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 233 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (A_T/A_S) \times (2/V)$

W_S: Amount (mg) of buformin hydrochloride 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 15 minutes of Buformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Buformin 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, and add water to make exactly V' mL so that each mL contains about 5.6 μg of buformin hydrochloride ($C_6H_{15}N_5$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water 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 233 nm.

Dissolution rate (%) with respect to the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S: Amount (mg) of buformin hydrochloride for assay
C: Labeled amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Buformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 60 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), add water to make exactly 200 mL, and treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution, centrifuge, pipet 2 mL of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 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 233 nm.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl)
$= W_S \times (A_T/A_S)$

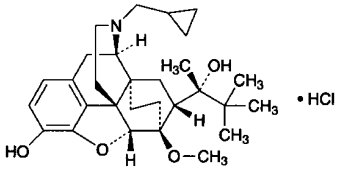
W_S: Amount (mg) of buformin hydrochloride for assay

Containers and storage Containers—Well-closed containers.

Add the following:

Buprenorphine Hydrochloride

ブプレノルフィン塩酸塩



$C_{29}H_{41}NO_4$.HCl: 504.10

(2*S*)-2-[(5*R*,6*R*,7*R*,14*S*)-17-(Cyclopropylmethyl)-4,5-epoxy-3-hydroxy-6-methoxy-6,14-ethanomorphinan-7-yl]-3,3-dimethylbutan-2-ol monohydrochloride [*53152-21-9*]

Buprenorphine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{29}H_{41}NO_4$.HCl.

Description Buprenorphine Hydrochloride occurs as white to yellowish white, crystals or a crystalline powder.

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

Melting point: about 268°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Buprenorphine Hydrochloride (1 in 5000) 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 Buprenorphine 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 Buprenorphine Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −92 – −98° (after drying, 0.4 g, methanol, 20 mL, 100 mm).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Buprenorphine Hydrochloride in 200 mL of water is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.1 g of Buprenorphine Hydrochloride in 10 mL of water is clear and colorless.

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

(3) Related substances—Dissolve 0.10 g of Buprenorphine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and 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 buprenorphine obtained from the sample solution is not larger than 1/4 times the peak area of buprenorphine from the standard solution. Furthermore, the total area of the peaks other than buprenorphine from the sample solution is not larger than 13/20 times the peak area of buprenorphine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

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

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

Mobile phase: A mixture of methanol, ammonium acetate solution (1 in 100), and acetic acid (100) (6000:1000:1).

Flow rate: Adjust the flow rate so that the retention time of buprenorphine is about 17 minutes.

Time span of measurement: About 2.5 times as long as the retention time of buprenorphine, 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 50 mL. Confirm that the peak area of buprenorphine obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buprenorphine are not less than 6500 and not more than 1.2, respectively.

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

Loss on drying <*2.41*> Not more than 1.0% (1 g, 115°C, 3 hours).

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

Assay Weigh accurately about 0.5 g of Buprenorphine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 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
= 50.41 mg of $C_{29}H_{41}NO_4.HCl$

Containers and storage Containers—Well-closed containers.

Calcium Chloride Injection

塩化カルシウム注射液

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 Membrane filtration method: it meets the requirement.

Calcium Folinate

ホリナートカルシウム

Change the Description and the Identification to read:

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

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

Identification (1) Determine the absorption spectrum of a solution of Calcium Folinate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Calcium Folinate 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 Calcium Folinate 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 Calcium Folinate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (2) and (3) for calcium salt.

Add the following next to Identification:

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +14 – +19° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH ⟨*2.54*⟩ To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the pH of this solution is between 6.8 and 8.0.

Change the Purity to read:

Purity (1) Clarity and color of solution—To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the solution is clear, and the absorbance at 420 nm of it, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.25.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 0.40 g of Calcium Folinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 10 mg of Calcium Folinate in 25 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 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 by the automatic integration method: the area of the peak other than folinate obtained from the sample solution is not larger than the peak area of folinate from the standard solution, and the total area of the peaks other than the peak of folinate from the sample solution is not larger than 5 times the peak area of folinate 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 2.5 times as long as the retention time of folinate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of folinate obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

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

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

Change the Water to read:

Water ⟨*2.48*⟩ Not less than 7.0% and not more than 17.0% (0.2 g, volumetric titration, direct titration).

Change the Assay to read:

Assay Weigh accurately about 10 mg each of Calcium Folinate and Calcium Folinate Reference Standard (separately determine the water ⟨*2.48*⟩ in the same manner as Calcium Folinate), dissolve in water to make them exactly 25 mL. Pipet 5 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Per-

form 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 folinate of both solutions.

$$\text{Amount (mg) of } C_{20}H_{21}CaN_7O_7 = W_S \times (A_T/A_S)$$

W_S: Amount (mg) of Calcium Folinate 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 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of disodium hydrogen phosphate dodecahydrate solution (287 in 100,000), methanol and tetrabutylammonium hydroxide TS (385:110:4), adjusted to pH7.5 with phosphoric acid.

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

System suitability—

System performance: Dissolve 10 mg each of Calcium Folinate and folic acid in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, folinate and folic acid 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 folinate is not more than 1.0%.

Camostat Mesilate

カモスタットメシル酸塩

Change the Identification (3) to read:

Identification **(3)** A 0.1 g portion of Camostat Mesilate responds to the Qualitative Tests <1.09> (1) for mesilate.

Add the following:

Cefadroxil Capsules

セファドロキシルカプセル

Cefadroxil Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

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

Identification Dissolve the contents of Cefadroxil Capsules, equivalent to 10 mg (potency) of Cefadroxil according to the labeled amount, in 500 mL of water, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

Water <2.48> Not more than 7.0% (0.15 g, 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.

Place 1 capsule of Cefadroxil Capsules in 300 mL of water, disperse with the aid of ultrasonic waves, shake for 30 minutes, and add water to make exactly 500 mL. Pipet 5 mL of this solution, and add water to make exactly V mL so that each mL contains about 0.1 mg (potency) of Cefadroxil. Filter the solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil Reference Standard, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

$$\text{Amount [mg (potency)] of cefadroxil } (C_{16}H_{17}N_3O_5S) = W_S \times (A_T/A_S) \times (V/2)$$

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

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 90 minutes of Cefadroxil Capsules is not less than 80%.

Start the test with 1 capsule of Cefadroxil 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 22 μg (potency) of Cefadroxil according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil Reference Standard, and add 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, at 263 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 cefadroxil ($C_{16}H_{17}N_3O_5S$)

$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

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

C: Labeled amount [mg (potency)] of cefadroxil in 1 capsule

Assay Take out the contents of 20 Cefadroxil Capsules, and combine. Weigh accurately the mass of the combined contents, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of Cefadroxil, add 300 mL of water, shake for 30 minutes, then add water to make exactly 500 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefadroxil Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= W_S \times (A_T/A_S) \times (5/2)$

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

Containers and storage Containers—Tight containers.

Add the following:

Cefadroxil for Syrup

シロップ用セファドロキシル

Cefadroxil for Syrup is a preparation for syrup which is suspended before use.

It contains not less than 95.0% and not more than 110.0% of the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of preparation Prepare as directed under Syrups, with Cefadroxil.

Identification Dissolve an amount of Cefadroxil for Syrup, equivalent to 10 mg (potency) of Cefadroxil according to the labeled amount, in 500 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

Water <*2.48*> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Uniformity of dosage units <*6.02*> The syrup in single-unit container 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 (put the sample in the dissolution medium so that it disperses), using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefadroxil for Syrup is not less than 85 %.

Start the test with accurately weighed amount of Cefadroxil for Syrup, equivalent to about 0.1 g (potency) of Cefadroxil 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 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 22 mg (potency) of Cefadroxil Reference Standard, 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, at 263 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$)

$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 450$

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

W_T: Amount (g) of sample

C: Labeled amount [mg (potency)] of cefadroxil in 1 g

Assay Weigh accurately an amount of powdered Cefadroxil for Syrup, equivalent to about 50 mg (potency) of Cefadroxil, dissolve in water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefadroxil Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= W_S \times (A_T/A_S) \times (5/2)$

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

Containers and storage Containers—Tight containers.

Cefalotin Sodium

セファロチンナトリウム

Change the origin/limits of content to read:

Cefalotin Sodium contains not less than 920 μg (potency) and not more than 980 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalotin Sodium is expressed as mass (potency) of cefalotin ($C_{16}H_{16}N_2O_6S_2$: 396.44).

Cefatrizine Propylene Glycolate

セファトリジンプロピレングリコール

Change the origin/limits of content to read:

Cefatrizine Propylene Glycolate contains not less than 816 μg (potency) and not more than 876 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefatrizine Propylene Glycolate is expressed as mass (potency) of cefatrizine ($C_{18}H_{18}N_6O_5S_2$: 462.50).

Add the following:

Cefazolin Sodium for Injection

注射用セファゾリンナトリウム

Cefazolin Sodium 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 amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Method of preparation Prepare as directed under Injections, with Cefazolin Sodium.

Description Cefazolin Sodium for Injection occurs as white to light yellowish white crystals or crystalline powder or masses.

Identification **(1)** Determine the absorption spectrum of a solution of Cefazolin Sodium for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 270 nm and 274 nm.

(2) Cefazolin Sodium for Injection responds to the Qualitative Tests <*1.09*> (1) for chloride.

Osmotic pressure ratio Being specified separately.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is 4.5 to 6.5.

Purity **(1)** Clarity and color of solution—Conduct this procedure within 10 minutes after the preparation of the solutions. A solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium according to the labeled amount, in 10 mL of water is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.35.

(2) Related substances—Dissolve an amount of Cefazolin Sodium for Injection, equivalent to 0.10 g (potency) of Cefazolin Sodium according to the labeled amount, in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak by the area percentage method: each area of the peaks other than cefazolin is not more than 1.5%. Furthermore the total area of the peaks other than cefazolin is not more than 2.5%. For these calculations, use the area of the peak, having the relative retention time of about 0.2 with respect to cefazolin, after multiplying by the relative response factor, 1.43.

Operating conditions—

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

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

System suitability—

Test for required detectability: Pipet 8 mL of the sample solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μL of this solution is equivalent to 3 to 7% of that from 5 μL of the solution for system suitability test.

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

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Water <*2.48*> Not more than 3.0% (0.5 g, volumetric titration, direct titration). Use a mixture of formamide for Karl Fischer method and methanol for Karl Fischer method (2:1) instead of methanol for Karl Fischer method.

Bacterial endotoxins <*4.01*> Less than 0.05 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 of the contents of not less than 10 containers of Cefazolin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Cefazolin Sodium, dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefazolin Reference Standard, equivalent to about 50 mg (potency), dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefazolin Sodium.

Amount [mg (potency)] of cefazolin ($C_{14}H_{14}N_8O_4S_3$) $= W_S \times (Q_T/Q_S)$

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

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Containers and storage Containers—hermetic containers. Plastic containers for aqueous injections may be used.

Add the following:

Cefmetazole Sodium for Injection

注射用セフメタゾールナトリウム

Cefmetazole Sodium 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 amount of cefmetazole ($C_{15}H_{17}N_7O_5S_3$: 471.53).

Method of preparation Prepare as directed under Injections, with Cefmetazole Sodium.

Description Cefmetazole Sodium for Injection is a white to light yellow powder or masses.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefmetazole Sodium for Injection (1 in 40,000) 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 Cefmetazole Sodium for Injection 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.

pH ⟨*2.54*⟩ Take an amount of Cefmetazole Sodium for Injection equivalent to 1.0 g (potency) of Cefmetazole Sodium according to the labeled amount, and dissolve in 10 mL of water: the pH of the solution is 4.2 to 6.2.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Cefmetazole Sodium for Injection, equivalent to 1.0 g (potency) of Cefmetazole Sodium according to the labeled amount, in 10 mL of water: the solution is clear and the color is not darker than the following control solution.

Control solution: Pipet 5 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, and add water to make exactly 50 mL. Pipet 15 mL of this solution, and add water to make exactly 20 mL.

(2) Related substances—Proceed as directed in the Purity (4) under Cefmetazole Sodium.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.06 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign particulate 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 Take 10 containers of Cefmetazole Sodium for Injection, dissolve the contents of each in the mobile phase, rinse each of the containers with the mobile phase, combine the rinse with the respective previous solution, and add the mobile phase to make exactly 500 mL. Take exactly a volume of this solution equivalent to about 0.2 g (potency) of Cefmetazole Sodium, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefmetazole Reference Standard, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefmetazole Sodium.

Amount [mg (potency)] of cefmetazole ($C_{15}H_{17}N_7O_5S_3$) $= W_S \times (Q_T/Q_S) \times 4$

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

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Add the following:

Ceftazidime for Injection

注射用セフタジジム

Ceftazidime 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 amount of ceftazidime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Method of preparation Prepare as directed under Injections, with Ceftazidime Hydrate.

Description Ceftazidime for Injection is a white to pale yellowish white powder.

Identification Determine the absorption spectrum of a solution of Ceftazidime for Injection (1 in 100,000) in phosphate buffer solution, pH 6.0, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 255 nm and 259 nm.

pH <*2.54*> Dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate according to the labeled amount, in 10 mL of water: the pH of this solution is 5.8 to 7.8.

Purity Clarity and color of solution—Dissolve 5 g of disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL. In 10 mL of this solution dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate according to the labeled amount: the solution is clear and colorless. Also, determine the absorption spectra of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 420 nm is not more than 0.3.

Loss on drying <*2.41*> Not more than 14.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 0.067 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 filter method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ceftazidime for Injection. Weigh accurately an amount of Ceftazidime Hydrate, equivalent to about 0.25 g (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add more 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ceftazidime Reference Standard, equivalent to about 25 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ceftazidime Hydrate.

Amount [mg (potency)] of ceftazidime ($C_{22}H_{22}N_6O_7S_2$)
$= W_S \times (Q_T/Q_S) \times 10$

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

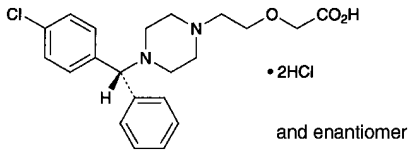
Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution, pH 7.0 (11 in 10,000).

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

Add the following:

Cetirizine Hydrochloride

セチリジン塩酸塩



$C_{21}H_{25}ClN_2O_3.2HCl$: 461.81
2-(2-{4-[(*RS*)-(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetic acid dihydrochloride [*83881-52-1*]

Cetirizine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{25}ClN_2O_3.2HCl$.

Description Cetirizine Hydrochloride occurs as a white crystalline powder.

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

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Cetirizine Hydrochloride (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Cetirizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) 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 Cetirizine 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 Cetirizine Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cetirizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Cetirizine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 50 mL. Pipet 5 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, and determine each peak area of each solution by the automatic integration method: the area of each peak other than cetirizine obtained from the sample solution is not larger than the peak area of cetirizine from the standard solution. Furthermore, the total area of the peaks other than cetirizine from the sample solution is not larger than 2.5 times the peak area of cetirizine from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.0 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 25°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L sulfuric acid TS (2 in 25) (47:3).

Flow rate: Adjust the flow rate so that the retention time of cetirizine is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of cetirizine, 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 10 mL. Confirm that the peak area of cetirizine obtained from 10 μL of this solution is equivalent to 35 to 65% of that from 10 μL of the standard solution.

System performance: Dissolve 20 mg of Cetirizine Hydrochloride in the mobile phase to make 100 mL. To 5 mL of this solution, add 3 mL of a solution of aminopyrine in the mobile phase (1 in 2500), and add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetirizine and aminopyrine 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 peak area of cetirizine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Cetirizine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetone and water (7:3), and titrate <*2.50*> to the second equivalence point 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
= 15.39 mg of $C_{21}H_{25}ClN_2O_3.2HCl$

Containers and storage Containers—Well-closed containers.

Add the following:

Cetirizine Hydrochloride Tablets

セチリジン塩酸塩錠

Cetirizine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$: 461.81).

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

Identification To a quantity of powdered Cetirizine Hydrochloride Tablets, equivalent to 10 mg of Cetirizine Hydrochloride according to the labeled amount, add about 70 mL of 0.1 mol/L hydrochloric acid TS, shake, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 4 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 230 nm and 234 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 1 tablet of Cetirizine Hydrochloride Tablets, add $4V/5$ mL of sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, treat with ultrasonic waves for 20 minutes, adjust the volume to exactly V mL, by adding sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, so that each mL contains about 0.2 mg of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$), 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 2 mL of the internal standard solution, add acetonitrile to make 10 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$)
$= W_S \times (Q_T/Q_S) \times (V/100)$

W_S: Amount (mg) of cetirizine hydrochloride for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 1000).

Assay Weigh accurately not less than 20 Cetirizine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$), add 40 mL of sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, treat with ultrasonic waves for 20 minutes, add sodium 1-heptanesulfonate solution (1 in 5000), adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, to make exactly 50 mL, and filter through a membrane 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 2 mL of the internal standard solution, add acetonitrile to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of cetirizine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours, and add sodium 1-heptanesulfonate solution (1 in 5000), adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add acetonitrile to make 10 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 cetirizine to that of the internal standard.

Amount (mg) of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$)
$= W_S \times (Q_T/Q_S) \times (1/2)$

W_S: Amount (mg) of cetirizine hydrochloride for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

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

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

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

Mobile phase: A mixture of a solution of sodium 1-heptansulfonate (1 in 2900) and acetonitrile (29:21), adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS.

Flow rate: Adjust the flow rate so that the retention time of cetirizine 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, cetirizine and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

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

Containers and storage Containers—Well-closed containers.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Add the following next to Purity:

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 procedures using light-resistant vessels. To 1 tablet of Chlordiazepoxide Tablets add 1 mL of water, shake to disintegrate the tablet, then add 20 mL of methanol, shake, add methanol to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, take exactly V mL of the subsequent filtrate equivalent to about 2 mg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), add exactly 1 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)
$= W_S \times (Q_T/Q_S) \times (5/V)$

W_S: Amount (mg) of Chlordiazepoxide Reference Standard

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Chlorphenesin Carbamate

クロルフェネシンカルバミン酸エステル

Change the Description to read:

Description Chlorphenesin Carbamate occurs as white crystals or a crystalline powder.

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

A solution of Chlorphenesin Carbamate in ethanol (95) (1 in 20) shows no optical rotation.

Change the Identification (2) to read:

Identification

(2) Determine the infrared absorption spectrum of

Chlorphenesin Carbamate, 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.

Change the Purity (3) to read:

Purity

(3) Chlorphenesin-2-carbamate—Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of hexane for liquid chromatography and 2-propanol (7:3), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak area, A_a, of chlorphenesin carbamate and the peak area, A_b, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, $A_b/(A_a+A_b)$, is not more than 0.007.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 5 mL of the solution for system suitability test add the mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 10 mL. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is equivalent to 40 to 60% of that of chlorphenesin carbamate obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in 50 mL of methanol. To 25 mL of this solution add 25 mL of dilute sodium hydroxide TS, and warm at 60°C for 20 minutes. To 20 mL of this solution add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethyl acetate, and allow to stand to separate the upper layer. When the procedure is run with 10 μL of this layer under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2-carbamate are eluted in this order, with the relative retention times of chlorphenesin and chlorphenesin-2-carbamate with respect to chlorphenesin carbamate being about 0.7 and about 1.2, respectively, and with the resolution between the peaks of chlorphenesin and chlorphenesin carbamate being not less than 2.0.

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

Add the following next to Purity (3):

(4) Related substances—Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 50 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 ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spot other than the principal spot from the sample solution is not more than one, and it is not more intense than the spot from the standard solution.

Add the following:

Chlorphenesin Carbamate Tablets

クロルフェネシンカルバミン酸エステル錠

Chlorphenesin Carbamate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$: 245.66).

Method of preparation Prepare as directed under Tablets, with Chlorphenesin Carbamate.

Identification To a quantity of powdered Chlorphenesin Carbamate Tablets, equivalent to 0.15 g of Chlorphenesin Carbamate according to the labeled amount, add 60 mL of ethanol (95), treat with ultrasonic waves, and add ethanol (95) to make 100 mL. Centrifuge 20 mL of this solution, add ethanol (95) to 1 mL of the supernatant liquid to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 226 nm and 230 nm, between 279 nm and 283 nm, and 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 Chlorphenesin Carbamate Tablets add 10 mL of water to disintegrate the tablet, add 70 mL of a mixture of water and methanol (1:1), treat with ultrasonic waves for 15 minutes with occasional stirring, then add the mixture of water and methanol (1:1) to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid equivalent to about 2.5 mg of chlorphenesin carbamate

($C_{10}H_{12}ClNO_4$), add the mixture of water and methanol (1:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 2 mL of this solution, add the mixture of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances at 280 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= W_S \times (A_T/A_S) \times (1/V) \times 5$

W_S: Amount (mg) of chlorphenesin carbamate 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 15 minutes of Chlorphenesin Carbamate Tablets is not less than 85%.

Start the test with 1 tablet of Chlorphenesin Carbamate 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 0.14 mg of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in 1 mL of methanol, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 450$

W_S: Amount (mg) of chlorphenesin carbamate for assay
C: Labeled amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Chlorphenesin Carbamate Tablets, and powder them in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 0.25 g of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$), add 30 mL of ethyl acetate, disperse using ultrasonic waves, then add ethyl acetate to make exactly 50 mL. Centrifuge 20 mL of this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add ethyl acetate to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in ethyl acetate to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, then add ethyl acetate to make 20 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 chlorphenesin carbamate to that of the internal standard.

Amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= W_S \times (Q_T/Q_S) \times (5/2)$

W_S: Amount (mg) of chlorphenesin carbamate for assay

Internal standard solution—A solution of ethenzamide in ethyl acetate (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—

System performance: Proceed as directed in the Purity (3) under Chlorphenesin Carbamate.

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 chlorphenesin carbamate to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Chlorpromazine Hydrochloride Injection

クロルプロマジン塩酸塩注射液

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 Membrane filtration method: it meets the requirement.

Chlorpromazine Hydrochloride Tablets

クロルプロマジン塩酸塩錠

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.

Conduct this procedures using light-resistant vessels. To 1 tablet of Chlorpromazine Hydrochloride Tablets add an amount of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) so that each mL contains about 0.83 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$), treat with the ultrasonic waves for 5 minutes, then shake vigorously for 20 minutes, and add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly V mL so that each mL contains about 0.5 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$). Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 2.5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$)
$= W_S \times (Q_T/Q_S) \times (V/50)$

W_S: Amount (mg) of chlorpromazine hydrochloride for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

Chlorpropamide Tablets

クロルプロパミド錠

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 Chlorpropamide Tablets add 75 mL of the mobile phase, treat with the ultrasonic waves for 20 minutes with occasional strong shaking, then add the mobile phase to make exactly V mL so that each mL contains about 2.5 mg of Chlorpropamide. Centrifuge the solution, pipet 2 mL of the supernatant liquid, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

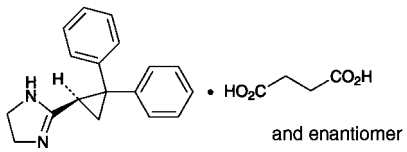
Amount (mg) of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$)
$= W_S \times (A_T/A_S) \times (V/20)$

W_S: Amount (mg) of chlorpropamide for assay

Add the following:

Cibenzoline Succinate

シベンゾリンコハク酸塩



$C_{18}H_{18}N_2.C_4H_6O_4$: 380.44
2-[(1*RS*)-2,2-Diphenylcyclopropan-1-yl]-4,5-dihydro-1*H*-imidazole monosuccinate [*100678-32-8*]

Cibenzoline Succinate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{18}H_{18}N_2.C_4H_6O_4$.

Description Cibenzoline Succinate occurs as a white crystalline powder.

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

A solution of Cibenzoline Succinate in methanol (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Cibenzoline Succinate (1 in 50,000) 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 Cibenzoline Succinate as directed in the paste 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) Shake 0.4 g of Cibenzoline Succinate with 2.5 mL of sodium hydroxide TS and 5 mL of ethyl acetate, allow to stand, and to 1 mL of the water layer so obtained add 0.5 mL of 1 mol/L hydrochloric acid TS and 0.5 mL of iron (III) chloride TS: a blown precipitate is formed.

Melting point ⟨*2.60*⟩ 163 – 167°C

pH ⟨*2.54*⟩ Dissolve 0.20 g of Cibenzoline Succinate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.20 g of Cibenzoline Succinate in 10 mL of water is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cibenzoline Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard

Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cibenzoline Succinate according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 25), and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cibenzoline Succinate 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 5 mL and 2 mL of this solution, add methanol to make them exactly 10 mL, and use these solutions as the standard solution (1) and 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 and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1). Allow the plate to stand for 30 minutes in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1), and the spot, which is more intense than the spot with the standard solution (2), is not more than two.

Loss on drying <*2.41*> Not more than 0.3% (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 Cibenzoline Succinate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from violet to blue-green through blue (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.04 mg of $C_{18}H_{18}N_2.C_4H_6O_4$

Containers and storage Containers—Tight containers.

Add the following:

Cibenzoline Succinate Tablets

シベンゾリンコハク酸塩錠

Cibenzoline Succinate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$: 380.44).

Method of preparation Prepare as directed under Tablets, with Cibenzoline Succinate.

Identification To a quantity of powdered Cibenzoline Succinate Tablets, equivalent to 50 mg of Cibenzoline Succinate according to the labeled amount, add 100 mL of water, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 221 nm and 225 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 Cibenzoline Succinate Tablets add a suitable amount of water so that each mL contains about 10 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), and allow standing for 10 minutes while occasional shaking. To this solution add methanol so that each mL contains about 2 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), add exactly 1 mL of the internal standard solution per 10 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), then add methanol so that each mL contains about 1 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$). Centrifuge the solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$)
$= W_S \times (Q_T/Q_S) \times (C/100)$

W_S: Amount (mg) of cibenzoline succinate for assay
C: Labeled amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) in 1 tablet

Internal standard solution—Dissolve 0.1 g of 2-ethylhexyl parahydroxybenzoate in methanol 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 Cibenzoline Succinate Tablets is not less than 80%.

Start the test with 1 tablet of Cibenzoline Succinate 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 cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of cibenzoline succinate for assay, previously dried at 105°C for 2 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. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 222 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of cibenzoline succinate for assay
C: Labeled amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) in 1 tablet

Assay Weigh accurately not less than 20 Cibenzoline Succinate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), add 10 mL of water, shake, and add 40 mL of methanol and exactly 10 mL of the internal standard solution. Shake for 20 minutes, add methanol to make 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of cibenzoline succinate for assay, previously dried at 105°C for 2 hours, add 10 mL of water and 40 mL of methanol to dissolve, then add exactly 10 mL of the internal standard solution and 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 cibenzoline to that of the internal standard.

Amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) $= W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of cibenzoline succinate for assay

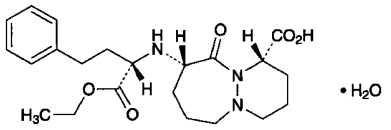
Internal standard solution—Dissolve 0.1 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 2.67 g of sodium di-2-ethylhexyl sulfosuccinate in 2000 mL of a mixture of water, acetonitrile and diluted phosphoric acid (1 in 10) (1000:1000:1).
Flow rate: Adjust the flow rate so that the retention time of cibenzoline is about 3 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cibenzoline 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 cibenzoline to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Cilazapril Hydrate

シラザプリル水和物



$C_{22}H_{31}N_3O_5.H_2O$: 435.51
(1*S*,9*S*)-9-[(1*S*)-(1-Ethoxycarbonyl-3-phenylpropyl)amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylic acid monohydrate [*92077-78-6*]

Cilazapril Hydrate contains not less than 98.5% and not more than 101.0% of cilazapril ($C_{22}H_{31}N_3O_5$: 417.50), calculated on the anhydrous basis.

Description Cilazapril Hydrate occurs as white to yellowish white crystals or crystalline powder.
It is very soluble in methanol, freely soluble in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.
It gradually turns yellow on exposure to light.
Melting point: about 101°C (with decomposition).

Identification **(1)** To 4 mL of a solution of Cilazapril Hydrate (1 in 1000) add 2 mL of Dragendorff's TS: an orange precipitate is produced.
(2) Determine the infrared absorption spectrum of Cilazapril 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*> $[\alpha]_D^{20}$: −53 – −58° (0.2 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Chloride <*1.03*>—Perform the test using 1.0 g of Cilazapril Hydrate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).
(2) Sulfate <*1.14*>—Dissolve 1.0 g of Cilazapril Hydrate in 40 mL of water and 1.5 mL of dilute hydrochloric 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 0.005 mol/L sulfuric acid VS (not more than 0.019%).
(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Cilazapril Hydrate according to Method 4, and perform the test. However, use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 8). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Related substances—Dissolve 0.10 g of Cilazapril Hydrate in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as

the standard solution (1). Pipet 3 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, pipet 2 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and three standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100), hexane, and water (62:15:10:10:3) to a distance of about 15 cm, and air-dry the plate. Leave the plate in iodine vapor for 2 hours, and examine the plate under ultraviolet light (main wavelength: 254 nm): of the spots other than the principal spot with an *R*f value close to 0.40 obtained from the sample solution, the spot in the vicinity of *R*f value 0.17 is not more intense than the spot from the standard solution (1), and the spot in the vicinity of *R*f value 0.44 is not more intense than the spot from the standard solution (2). The number of all other spot does not exceed 3, and of these spots, no more than one is more intense than the spot from the standard solution (3) and none are more intense than the spot from the standard solution (2).

Water <*2.48*> 3.5 – 5.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Cilazapril Hydrate, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 8.350 mg of $C_{22}H_{31}N_3O_5$

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

Add the following:

Cilazapril Tablets

シラザプリル錠

Cilazapril Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of cilazapril ($C_{22}H_{31}N_3O_5$: 417.50).

Method of preparation Prepare as directed under Tablets, with Cilazapril Hydrate.

Identification To a quantity of powdered Cilazapril Tablets, equivalent to 2 mg of cilazapril ($C_{22}H_{31}N_3O_5$) according to the labeled amount, add 2 mL of a mixture of acetonitrile and ethyl acetate (3:1), shake, treat with ultrasonic waves for 30 seconds, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 5 mg of cilazapril in 5 mL of the mixture of acetonitrile and ethyl acetate (3:1), 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 ethyl acetate, methanol, acetic acid (100), hexane and water (62:15:10:10:3) to a distance of about 15 cm, and air-dry the plate. Place the plate in iodine vapor for 2 hours, and immediately examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample and standard solutions are dark brown and they 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 Cilazapril Tablets add 5 mL of a mixture of water and acetonitrile (7:3), shake well until disintegration, add the mixture of water and acetonitrile (7:3) to make exactly *V* mL so that each mL contains about 25 μg of cilazapril ($C_{22}H_{31}N_3O_5$), and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of cilazapril for assay (separately determine the water <*2.48*> in the same manner as Cilazapril Hydrate), and dissolve in the mixture of water and acetonitrile (7:3) to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 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 calculate the ratios, Q_T and Q_S, of the peak area of cilazapril to that of the internal standard.

Amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$)
$= W_S \times (Q_T/Q_S) \times (V/1000)$

W_S: Amount (mg) of cilazapril for assay, calculated on the anhydrous basis

Internal standard solution—A solution of dimethyl phthalate in a mixture of water and acetonitrile (7:3) (1 in 12,500).
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 conditions, cilazapril 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 100 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of cilazapril 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 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cilazapril Tablets is not less than 85%.

Start the test with 1 tablet of Cilazapril 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, and add water to make exactly V' mL so that each mL contains about 0.28 μg of cilazapril ($C_{22}H_{31}N_3O_5$) according to the labeled amount. Pipet 10 mL of the solution, add exactly 5 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 29 mg of cilazapril for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Cilazapril Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 100 mL. Then, pipet 2 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of acetonitrile, 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, and determine the peak areas of cilazapril, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of cilazapril ($C_{22}H_{31}N_3O_5$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/10)$$

W_S: Amount (mg) of cilazapril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$) in 1 tablet

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: To a solution consisting of 180 mL of tetrahydrofuran for liquid chromatography, 120 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine add water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cilazapril is about 10 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 cilazapril 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 conditions, the relative standard deviation of the peak area of cilazapril is not more than 2.0%.

Assay Weigh acurately 20 Cilazapril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of cilazapril ($C_{22}H_{31}N_3O_5$), add 30 mL of a mixture of water and acetonitrile (7:3), and treat with ultrasonic waves for 5 minutes. Next, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 50 mL, and centrifuge. Filter the supernatant liquid 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 26 mg of cilazapril for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Cilazapril Hydrate), and dissolve in the mixture of water and acetonitrile (7:3) to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 50 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 cilazapril to that of the internal standard.

Amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$)

$$= W_S \times (Q_T/Q_S) \times (1/25)$$

W_S: Amount (mg) of cilazapril for assay, calculated on the anhydrous basis

Internal standard solution—A solution of dimethyl phthalate in a mixture of water and acetonitrile (7:3) (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 23°C.

Mobile phase: To a solution consisting of 180 mL of tetrahydrofuran for liquid chromatography, 120 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine add water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid.

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

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above conditions, cilazapril 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 50 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of cilazapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cilostazol Tablets

シロスタゾール錠

Change the Assay to read:

Assay Weigh accurately not less than 20 Cilostazol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of cilostazol ($C_{20}H_{27}N_5O_2$), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and shake well for 10 minutes. To 1 mL of this solution add methanol to make 10 mL, filter 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 50 mg of Cilostazol Reference Standard, previously dried at 105°C for 2 hours, dissolve in a suitable amount of methanol, and add exactly 5 mL of the internal standard solution, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and 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 cilostazol to that of the internal standard.

Amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) = $W_S \times (Q_T/Q_S)$

W_S: Amount (mg) of Cilostazol Reference Standard

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cilostazol.

System suitability—

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

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 cilostazol to that of the internal standard is not more than 1.5%.

Add the following:

Clindamycin Phosphate Injection

クリンダマイシンリン酸エステル注射液

Clindamycin Phosphate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$: 504.96).

Method of preparation Prepare as directed under Injections, with Clindamycin Phosphate.

Description Clindamycin Phosphate Injection is a clear, colorless or light yellow liquid.

Identification To a volume of Clindamycin Phosphate Injection, equivalent to 0.15 g (potency) of Clindamycin Phosphate according to the labeled amount, add 4 mL of water, 2 mL of 8 mol/L sodium hydroxide TS and 0.1 mL of sodium pentacyanonitrosylferrate (III) TS, mix, heat in a water bath for 10 minutes, and add 2 mL of hydrochloric acid: a blue-green color develops.

Osmotic pressure ratio Being specified separately.

pH <*2.54*> 6.0 – 7.0

Bacterial endotoxins <*4.01*> Less than 0.1 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 Measure exactly a volume of Clindamycin Phosphate Injection, equivalent to about 0.3 g (potency) of Clindamycin Phosphate, and add the mobile phase to make exactly 100 mL. Pipet 7 mL of this solution, add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Clindamycin Phosphate Reference Standard, equivalent to about 20 mg (potency), dissolve in exactly 25 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 Clindamycin Phosphate.

Amount [mg (potency)] of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$)
= $W_S \times (Q_T/Q_S) \times (100/7)$

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

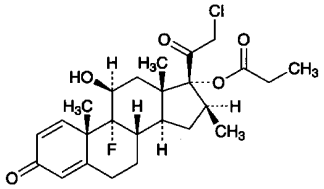
Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

Containers and storage Containers—Hermetic containers.

Add the following:

Clobetasol Propionate

クロベタゾールプロピオン酸エステル



$C_{25}H_{32}ClFO_5$: 466.97
21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propanoate
[*25122-46-7*]

Clobetasol Propionate, when dried, contains not less than 97.0% and not more than 102.0% of $C_{25}H_{32}ClFO_5$.

Description Clobetasol Propionate occurs as a white to pale yellowish white crystalline powder.

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

It gradually turns yellow by light.

Melting point: about 196°C (with decomposition).

Identification Determine the infrared absorption spectra of Clobetasol Propionate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Clobetasol Propionate Reference Standard: both spectra exhibit similar intensities of absorbance at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +109 – +115° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Clobetasol Propionate 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 10 mg of Clobetasol Propionate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 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 these solutions by the automatic integration method: the area of the peak other than clobetasol propionate obtained from the sample solution is not larger than 2/5 times the peak area of clobetasol propionate from the standard solution. Furthermore, the total area of the peaks other than clobetasol propionate from the sample solution is not larger than the peak area of clobetasol propionate from the standard solution.

Operating conditions—

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

Time span of measurement: About 2.5 times as long as the retention time of clobetasol propionate, 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 50 mL. Confirm that the peak area of clobetasol propionate obtained from 10 μL of this solution is equivalent to 2.8 to 5.2 % of that from 10 μL of the standard solution.

System performance: Dissolve 20 mg of Clobetasol Propionate in 20 mL of methanol. To 5 mL of this solution add 10 mL of a solution of beclometasone dipropionate in methanol (1 in 1000), and then add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above conditions, clobetasol propionate and beclometasone dipropionate 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 conditions, the relative standard deviation of the peak area of clobetasol propionate is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 10 mg each of Clobetasol Propionate and Clobetasol Propionate Reference Standard, both previously dried, dissolve each in the mobile phase, add exactly 100 mL of the internal standard solution, add the mobile phase to make 250 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 clobetasol propionate to that of the internal standard.

$$\text{Amount (mg) of } C_{25}H_{32}ClFO_5 = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Clobetasol Propionate Reference Standard

Internal standard solution—A solution of beclometasone dipropionate in the mobile phase (1 in 5000).

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 25°C.

Mobile phase: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.5 with phosphoric acid, and then add water to make 1000 mL. To 425 mL of this solution add 475 mL of acetonitrile and 100 mL of methanol.

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above conditions, clobetasol propionate 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 conditions, the relative standard deviation of the ratio of the peak area of clobetasol propionate to that of the internal standard is not more than 1.0%.

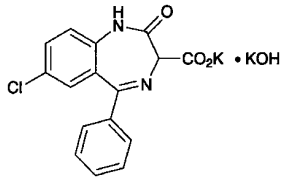
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Clorazepate Dipotassium

クロラゼプ酸二カリウム



$C_{16}H_{10}ClKN_2O_3.KOH$: 408.92

Monopotassium 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine-3-carboxylate mono (potassium hydroxide) [*57109-90-7*]

Clorazepate Dipotassium, when dried, contains not less than 98.5% and not more than 101.0% of $C_{16}H_{10}ClKN_2O_3.KOH$.

Description Clorazepate Dipotassium occurs as white to light yellow, crystals or crystalline powder.

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

It dissolves in acetic acid (100).

The pH of a solution obtained by dissolving 1 g of Clorazepate Dipotassium in 100 mL of water is between 11.5 and 12.5.

It gradually turns yellow on exposure to light.

Identification (1) Carefully and gradually ignite to redness 30 mg of Clorazepate Dipotassium with 50 mg of sodium. After cooling, add 3 drops of ethanol (99.5) and 5 mL of water, mix well, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for chloride.

(2) Determine the absorption spectrum of a solution of Clorazepate Dipotassium (1 in 200,000) 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 Clorazepate Dipotassium 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.

(4) Clorazepate Dipotassium responds to the Qualitative Tests <*1.09*> (1) for potassium salt.

Purity (1) Chloride <*1.03*>—Dissolve 1.0 g of Clorazepate Dipotassium in 20 mL of water, add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with 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 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.014 %).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Clorazepate Dipotassium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Clorazepate Dipotassium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 15 mg of Clorazepate Dipotassium in 25 mL of a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Prepare these solutions quickly and perform the test within 3 minutes. 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 each peak area by the automatic integration method: the peak area of nordiazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, obtained from the sample solution is not larger than the peak area of clorazepic acid from the standard solution, the area of the peak other than clorazepic acid and nordiazepam is not larger than 1/5 times the peak area of clorazepic acid from the standard solution, and the total area of the peaks other than clorazepic acid from the sample solution is not larger than 2 times the peak area of clorazepic acid from the standard solution. For this comparison, use the peak area of nordiazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, after multiplying by the relative response factor, 0.64.

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 25°C.

Mobile phase: Dissolve 13.8 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, and adjust to pH 8.0 with sodium hydroxide TS. To 100 mL of this solution add 400 mL of acetonitrile and 300 mL of water.

Flow rate: Adjust the flow rate so that the retention time of clorazepic acid is about 1.3 minutes.

Time span of measurement: About 10 times as long as the retention time of clorazepic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 25 mL. Confirm that the peak area of clorazepic acid obtained from 5 μL of this solution is equivalent to 15 to 25 % of that from 5 μL of 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 clorazepic acid 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 clorazepic acid is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

Assay Weigh accurately about 0.15 g of Clorazepate Dipotassium, previously dried, dissolve in 100 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of solution changes from violet to blue-green through blue (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.63 mg of $C_{16}H_{10}ClKN_2O_3$.KOH

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Clorazepate Dipotassium Capsules

クロラゼプ酸二カリウムカプセル

Clorazepate Dipotassium Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH: 408.92).

Method of preparation Prepare as directed under Capsules, with Clorazepate Dipotassium.

Identification To 10 mL of the sample solution obtained in the Assay add water to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 228 nm and 232 nm.

Purity Related substances—Take out the contents of Clorazepate Dipotassium Capsules, and powder. To a portion of the powder, equivalent to 15 mg of Clorazepate Dipotassium according to the labeled amount, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make 25 mL, and mix for 10 minutes. Filter the 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. Pipet 1 mL of the sample solution, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Purity (4) under Clorazepate Dipotassium: the peak area of nordiazepam, having the relative retention time of about 3.0 with respect to clorazepic acid, obtained from the sample solution is not larger than 3 times the peak area of clorazepic acid from the standard solution, and the total area of the peaks other than clorazepic acid and nordiazepam from the sample solution is not larger than the peak area of clorazepic acid from the standard solution. For this comparison, use the peak area of nordiazepam after multiplying by the relative response factor, 0.64.

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 Clorazepate Dipotassium Capsules add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet V mL of the supernatant liquid, add water to make exactly V' mL so that each mL contains about 12 μg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH)
$= W_S \times (A_T/A_S) \times (V'/V) \times (2/25)$

W_S: Amount (mg) of clorazepate dipotassium 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 30 minutes of Clorazepate Dipotassium Capsules is not less than 80%.

Start the test with 1 capsule of Clorazepate Dipotassium 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 8.3 μg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 21 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 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, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of clorazepate dipotassium for assay
C: Labeled amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH) in 1 capsule

Assay Carefully take out the contents of not less than 20 Clorazepate Dipotassium Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH), add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet 4 mL of the supernatant liquid, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water 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 252 nm.

Amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of clorazepate dipotassium for assay

Containers and storage Containers—Tight containers.

Creosote

Change the title of the monograph as follows:

Wood Creosote

木クレオソート

Cyanocobalamin

シアノコバラミン

Change the Identification (2) and (3) to read:

Identification

(2) Mix 1 mg of Cyanocobalamin with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a 50-mL distilling flask, dissolve in 5 mL of water, and add 2.5 mL of hypophosphorous acid. Connect the flask with a short condenser, and dips its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distil 1 mL into a test tube. To the test tube add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 30 mg of sodium fluoride, and heat the contents to boil. Immediately add dropwise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

Change the Purity (2) to read:

Purity

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 10 mg of Cyanocobalamin in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peak other than cyanocobalamin obtained from the sample solution is not larger than the peak area of cyanocobalamin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 361 nm).

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

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 10 g of anhydrous disodium hydrogen phosphate in 1000 mL of water, and adjust to pH 3.5 with phosphoric acid. To 147 mL of this solution add 53

mL of methanol.

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

Time span of measurement: About 4 times as long as the retention time of cyanocobalamin, 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 cyanocobalamin obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the solution for system suitability test.

System performance: Perform this procedure quickly after the solution is prepared. To 25 mg of cyanocobalamin add 10 mL of water, and warm, if necessary, to dissolve. After cooling, add 0.5 mL of sodium toluenesulfonchloramide TS, 0.5 mL of 0.05 mol/L hydrochloric acid TS and water to make 25 mL, mix, and allow the solution to stand for 5 minutes. To 1 mL of the solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of the solution under the above operating conditions, two principal peaks appear 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cyanocobalamin is not more than 3.0%.

Cyanocobalamin Injection

シアノコバラミン注射液

Change the Description to read:

Description Cyanocobalamin Injection is a clear, light red to red liquid.

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.30 EU/μg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

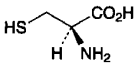
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

L-Cysteine

L-システイン



$C_3H_7NO_2S$: 121.16

(2*R*)-2-Amino-3-sulfanylpropanoic acid [*52-90-4*]

L-Cysteine contains not less than 98.5% and not more than 101.0% of $C_3H_7NO_2S$, calculated on the dried basis.

Description L-Cysteine occurs as white crystals or a white crystalline powder. It has a characteristic odor and a pungent taste.

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

It dissolves in 1 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Cysteine 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*⟩ $[\alpha]_D^{20}$: +8.0 – +10.0° (2 g calculated on the dried basis, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.25 g of L-Cysteine in 50 mL of water is 4.7 to 5.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Cysteine in 20 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.30 g of L-Cysteine in 10 mL of diluted nitric acid (1 in 4), add 10 mL of hydrogen peroxide (30), heat for 20 minutes in a boiling water bath, cool, and then add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Cysteine in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. Prepare the test solution and the control solution with 4 mL of barium chloride TS, respectively (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Cysteine, using the distillation under reduced pressure. 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-Cysteine 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-Cysteine according to Method 1, and perform the test using Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Cysteine in *N*-ethylmaleimide solution (1 in 50) to make 10 mL, leave for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution (1). Separately, dissolve 0.10 g of L-cystine in 0.5 mol/L hydrochloric acid TS to make 20 mL. Pipet 1 mL of this solution, add water to make 100 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 and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate for 30 minutes at 80°C. Spray the plate evenly with a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100), and then heat at 80°C for 10 minutes: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (2) is not more intense than the spot from the standard solution (2). Also, the spots other than the principal spot and the spots mentioned above from the sample solution are not more intense than the spot from the standard solution (1).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of L-Cysteine, place it in a stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, immediately place in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, leave in a dark place for 20 minutes, and then titrate ⟨*2.50*⟩ an excess amount of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination using the same method.

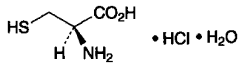
Each mL of 0.05 mol/L iodine VS = 12.12 mg of $C_3H_7NO_2S$

Containers and storage Containers—Tight containers.

Add the following:

L-Cysteine Hydrochloride Hydrate

L-システイン塩酸塩水和物



$C_3H_7NO_2S.HCl.H_2O$: 175.63

(2*R*)-2-Amino-3-sulfanylpropanoic acid monohydrochloride monohydrate [*7048-04-6*]

L-Cysteine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of L-cysteine hydrochloride ($C_3H_7NO_2S.HCl$: 157.62), calculated on the dried basis.

Description L-Cysteine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a characteristic odor and a strong acid taste.

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

It dissolves in 6 mol/L hydrochloric acid TS.

Identification (1) Determine the infrared absorption spectrum of L-Cysteine Hydrochloride Hydrate 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) To 10 mL of a solution of L-Cysteine Hydrochloride Hydrate (1 in 50) add 1 mL of hydrogen peroxide (30), heat on a water bath for 20 minutes, and cool: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +6.0 – +7.5° (2 g, calculated on the dried basis, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 100 mL of water is between 1.3 and 2.3.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 10 mL of water is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Dissolve 0.8 g of L-Cysteine Hydrochloride Hydrate in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. To both of the test solution and the control solution add 4 mL of barium chloride TS (not more than 0.021%).

(3) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Cysteine Hydrochloride Hydrate using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of

L-Cysteine Hydrochloride Hydrate 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).

(5) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Cysteine Hydrochloride Hydrate 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).

(6) Related substances—Dissolve 0.10 g of L-Cysteine Hydrochloride Hydrate in *N*-ethylmaleimide solution (1 in 50) to make 10 mL, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under 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. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Loss on drying ⟨*2.41*⟩ 8.5 – 12.0% (1 g, in vacuum, phosphorus (V) oxide, 20 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of L-Cysteine Hydrochloride Hydrate, place in a glass-stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, soak immediately in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, allow to stand for 20 minutes in a dark place, titrate ⟨*2.50*⟩ the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 15.76 mg of $C_3H_7NO_2S.HCl$

Containers and storage Containers—Tight containers.

Deferoxamine Mesilate

デフェロキサミンメシル酸塩

Change the Identification (2) to read:

Identification

(2) A 50 mg portion of Deferoxamine Mesilate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for mesilate.

Dehydrocholic Acid Injection

デヒドロコール酸注射液

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 Membrane filtration method: it meets the requirement.

Deslanoside Injection

デスラノシド注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 500 EU/mg.

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.

Dextran 40

デキストラン **40**

Delete the Pyrogen and add the following next to Residue on ignition:

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.5 EU/g.

Change to read:

Anhydrous Dibasic Calcium Phosphate

無水リン酸水素カルシウム

$CaHPO_4$: 136.06
[*7757-93-9*]

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

Anhydrous Dibasic Calcium Phosphate contains not less than 98.0% and not more than 103.0% of $CaHPO_4$.

◆**Description** Anhydrous Dibasic Calcium Phosphate occurs as white, crystalline powder or granules.

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

It dissolves in dilute hydrochloric acid and in dilute nitric acid.◆

Identification **(1)** Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

Purity **(1)** Acid-insoluble substances—Dissolve 5.0 g of Anhydrous Dibasic Calcium Phosphate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity of the washings is produced when silver nitrate TS is added. Ignite to incinerate the residue and the filter paper at 600 ± 50°C: the mass is not more than 10 mg (not more than 0.2%).

(2) Chloride <*1.03*>—Dissolve 0.20 g of Anhydrous Dibasic Calcium Phosphate in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of 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 0.248%).

(3) Sulfate <*1.14*>—Dissolve 0.5 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 20 mL of this solution, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.48%).

(4) Carbonate—Mix 1.0 g of Anhydrous Dibasic Calcium Phosphate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

◆**(5)** Heavy metals <*1.07*>—Dissolve 0.65 g of Anhydrous Dibasic Calcium Phosphate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).◆

(6) Barium—Heat 0.5 g of Anhydrous Dibasic Calcium Phosphate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

◆**(7)** Arsenic <*1.11*>—Dissolve 1.0 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).◆

Loss on ignition <*2.43*> Not less than 6.6% and not more than 8.5% (1 g, 800 – 825°C, constant mass).

Assay Weigh accurately about 0.4 g of Anhydrous Dibasic Calcium Phosphate, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> the excess of disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.721 mg of $CaHPO_4$

◆**Containers and storage** Containers—Well-closed containers.◆

Change to read:

Dibasic Calcium Phosphate Hydrate

リン酸水素カルシウム水和物

$CaHPO_4.2H_2O$: 172.09
[*7789-77-7*]

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

Dibasic Calcium Phosphate Hydrate contains not less than 98.0% and not more than 105.0% of $CaHPO_4.2H_2O$.

◆**Description** Dibasic Calcium Phosphate Hydrate occurs as a white, crystalline powder.

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

It dissolves in dilute hydrochloric acid and in dilute nitric acid.◆

Identification **(1)** Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

Purity **(1)** Acid-insoluble substance—Dissolve 5.0 g of Dibasic Calcium Phosphate Hydrate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity of the washing is produced when silver nitrate TS is added. Ignite to incinerate the residue and filter paper at 600 ± 50°C: the mass is not more than 10 mg (not more than 0.2%).

(2) Chloride <*1.03*>—Dissolve 0.20 g of Dibasic Calcium Phosphate Hydrate in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of 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 0.248%).

(3) Sulfate <*1.14*>—Dissolve 0.5 g of Dibasic Calcium Phosphate Hydrate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 20 mL of this solution, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.48%).

(4) Carbonate—Mix 1.0 g of Dibasic Calcium Phosphate Hydrate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

◆**(5)** Heavy metals <*1.07*>—Dissolve 0.65 g of Dibasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).◆

(6) Barium—Heat 0.5 g of Dibasic Calcium Phosphate Hydrate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

◆**(7)** Arsenic <*1.11*>—Dissolve 1.0 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).◆

Loss on ignition <*2.43*> Not less than 24.5% and not more than 26.5% (1 g, 800 – 825°C, constant mass).

Assay Weigh accurately about 0.4 g of Dibasic Calcium Phosphate Hydrate, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> the excess of disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

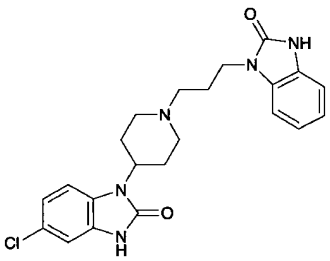
Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 3.442 mg of $CaHPO_4.2H_2O$

◆**Containers and storage** Containers—Well-closed containers.◆

Add the following:

Domperidone

ドンペリドン



$C_{22}H_{24}ClN_5O_2$: 425.91

5-Chloro-1-{1-[3-(2-oxo-2,3-dihydro-1*H*-benzoimidazol-1-yl)propyl]piperidin-4-yl}-1,3-dihydro-2*H*-benzoimidazol-2-one [*57808-66-9*]

Domperidone, when dried, contains not less than 99.0% and not more than 101.0% of $C_{22}H_{24}ClN_5O_2$.

Description Domperidone occurs as a white to pale yellow, crystalline powder or powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), very slightly soluble in 2-propanol, and practically insoluble in water.

Melting point: about 243°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Domperidone in a mixture of 2-propanol and 0.1 mol/L hydrochloric acid TS (9:1) (1 in 50,000) 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 Domperidone 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 2.0 g of Domperidone 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 Domperidone in 100 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 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 each peak area of each solution by the automatic integration method: the area of each peak other than domperidone obtained from the sample solution is not larger than 1/2 times the peak area of domperidone from the standard solution. Furthermore, the total area of the peaks other than domperidone from the sample solution is not larger than the peak area of domperidone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 287 nm).

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

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

Mobile phase: Dissolve 2.72 g of dipotassium hydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 of this solution with a solution prepared by dissolving 2.31 g of phosphoric acid in water to make 1000 mL. To 500 mL of this solution add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of domperidone is about 9 minutes.

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

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add methanol to make exactly 5 mL. Confirm that the peak area of domperidone obtained from 10 μL of this solution is equivalent to 30 to 50% of that from 10 μL of the standard solution.

System performance: Dissolve 10 mg of Domperidone and 20 mg of ethyl parahydroxybenzoate in 100 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, domperidone and ethyl parahydroxybenzoate 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 domperidone is not more than 3.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 0.5 g of Domperidone, 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
= 42.59 mg of $C_{22}H_{24}ClN_5O_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dopamine Hydrochloride Injection

ドパミン塩酸塩注射液

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 Membrane filtration method: it meets the requirement.

Add the following:

Doxorubicin Hydrochloride for Injection

注射用ドキソルビシン塩酸塩

Doxorubicin Hydrochloride 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 amount of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl: 579.98).

Method of preparation Prepare as directed under Injections, with Doxorubicin Hydrochloride.

Description Doxorubicin Hydrochloride for Injection occurs as red-orange, powder or masses.

Identification Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 10 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount, in methanol to make 100 mL. To 5 mL of this solution add methanol to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 231 nm and 235 nm, between 250 nm and 254 nm, between 477 nm and 481 nm, and between 493 nm and 497 nm, and exhibits a shoulder between 528 nm and 538 nm.

pH ⟨*2.54*⟩ The pH of a solution, prepared by dissolving an amount of Doxorubicin Hydrochloride for Injection equivalent to 10 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount in 2 mL of water, is 5.0 to 6.0.

Purity Clarity and color of solution—Dissolve an amount of Doxorubicin Hydrochloride for Injection, equivalent to 50 mg (potency) of Doxorubicin Hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and red.

Water ⟨*2.48*⟩ Not more than 4.0% (0.25 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.50 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 of the contents of not less than 10 containers of Doxorubicin Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Doxorubicin Hydrochloride, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use the solution as the sample solution. Separately, weigh accurately an amount of Doxorubicin Hydrochloride Reference Standard, equivalent to 10 mg (potency), add exactly 5 mL of the internal standard solution and the mobile phase 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*⟩, and calculate the ratios, Q_T and Q_S, of the peak area of doxorubicin to that of the internal standard.

Amount [mg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl)
$= W_S \times (Q_T/Q_S)$

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

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (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 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000). To this solution add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin 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, doxorubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5, and the symmetry factor of the peak of doxorubicin is between 0.8 and 1.2.

System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of doxorubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Edrophonium Chloride Injection

エドロホニウム塩化物注射液

Add the following next to pH:

Bacterial endotoxins ⟨*4.01*⟩ Less than 15 EU/mg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

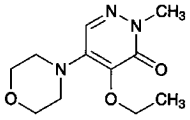
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Emorfazone

エモルファゾン



$C_{11}H_{17}N_3O_3$: 239.27

4-Ethoxy-2-methyl-5-(morpholin-4-yl)pyridazin-3(2*H*)-one [*38957-41-4*]

Emorfazone, when dried, contains not less than 98.5 % and not more than 101.0% of $C_{11}H_{17}N_3O_3$.

Description Emorfazone occurs as colorless crystals or a white to light yellow crystalline powder.

It is very soluble in ethanol (99.5), and freely soluble in water and in acetic anhydride.

It dissolves in 1 mol/L hydrochloric acid TS.

It gradually turns yellow and decomposes on exposure to light.

Identification **(1)** Dissolve 20 mg of Emorfazone in 2 mL of 1 mol/L hydrochloric acid TS, and add 5 drops of Reinecke's TS: light red floating matters are formed.

(2) Determine the absorption spectrum of a solution of Emorfazone (1 in 100,000) 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 Emorfazone 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*⟩ 89 – 92°C (after drying).

Purity **(1)** Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of Emorfazone. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018 %).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Emorfazone 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 2.0 g of Emorfazone according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.5 g of Emorfazone in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 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 by the automatic integration method: each peak area other than emorfazone obtained from the sample solution is not larger than 1/10 times the peak area of emorfazone from the standard solution, and the total area of the peaks other than the peak of emorfazone from the sample solution is not larger than 1/2 times the peak area of emorfazone 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 25°C.

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.

Time span of measurement: About 2.5 times as long as the retention time of emorfazone, beginning after the solvent peak.

System suitability—

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

System performance: Dissolve 16 mg of Emorfazone and 30 mg of 2,4-dinitrophenylhydrazine in 100 mL of

methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, emorfazone and 2,4-dinitrophenylhydrazine are eluted in this order with the resolution between these peaks being not less than 2.5.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Emorfazone, previously dried, dissolve in 60 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.

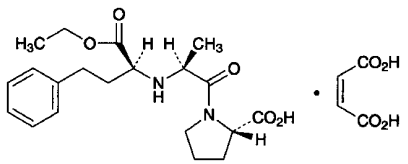
Each mL of 0.1 mol/L perchloric acid VS
= 23.93 mg of $C_{11}H_{17}N_3O_3$

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

Add the following:

Enalapril Maleate

エナラプリルマレイン酸塩



$C_{20}H_{28}N_2O_5.C_4H_4O_4$: 492.52
(2*S*)-1-{(2*S*)-2-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropylamino]propanoyl}pyrrolidine-2-carboxylic acid monomaleate [*76095-16-4*]

Enalapril Maleate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{20}H_{28}N_2O_5.C_4H_4O_4$.

Description Enalapril Maleate occurs as white crystals or a white crystalline powder.

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

Melting point: about 145°C (with decomposition).

Identification (1) Determine the infrared absorption spectra of Enalapril Maleate as directed in the potassium bromide disc method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Enalapril Maleate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 20 mg of Enalapril Maleate add 5 mL of 1 mol/L hydrochloric acid TS, shake, add 5 mL of diethyl ether, and shake for 5 minutes. Take 3 mL of the upper layer, distil off the diethyl ether on a water bath, add 5 mL of water to the residue with shaking, and add 1 drop of potassium permanganate TS: the red color of the test solution immediately disappears.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −41.0 – −43.5° (after drying, 0.25 g, methanol, 25 mL, 100 mm).

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Enalapril Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Enalapril Maleate in 100 mL of a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) 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*⟩ acccording to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than maleic acid and enalapril obtained from the sample solution is not larger than the peak area of enalapril from the standard solution. Furthermore, the total area of the peaks other than maleic acid and enalapril from the sample solution is not larger than twice the peak area of enalapril from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phases, mobile phase flow, 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 enalapril, beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril 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 conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Enalapril Maleate and Enalapril Maleate Reference Standard, both previously dried, and dissolve in a mixture of sodium dihydrogen phosphate TS, pH 2.5 and acetonitrile (19:1) to make exactly 100 mL, and use these solutions as the sample solution and 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 of enalapril, A_T and A_S, of both solutions.

$$\text{Amount (mg) of } C_{20}H_{28}N_2O_5.C_4H_4O_4 = W_S \times (A_T/A_S)$$

W_S: Amount (mg) of Enalapril Maleate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 15 cm in length, packed with porous styrene-divinylbenzene copolymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase A: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 950 mL of this solution, add 50 mL of acetonitrile for liquid chromatography.

Mobile phase B: Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 6.8 with a solution of sodium hydroxide (1 in 4), and add water to make 1000 mL. To 340 mL of this solution, add 660 mL of acetonitrile for liquid chromatography.

Mobile phase flow: Control the concentration gradient by changing the ratio of the mobile phases A and B as follows.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0	95	5
0 – 20	95 → 40	5 → 60
20 – 25	40	60

Flow rate: 1.4 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril 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 conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Enalapril Maleate Tablets

エナラプリルマレイン酸塩錠

Enalapril Maleate Tablets contain not less than 93.0 % and not more than 107.0% of the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$: 492.52).

Method of preparation Prepare as directed under Tablets, with Enalapril Maleate.

Identification To a quantity of powdered Enalapril Maleate Tablets equivalent to 50 mg of Enalapril Maleate according to the labeled amount, add 20 mL of methanol, shake, centrifuge, and then use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of enalapril maleate 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 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 water, acetone, 1-butanol, acetic acid (100) and toluene (1:1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f values of the 2 spots obtained from the sample solution and the 2 spots obtained from the standard solution are equivalent.

Purity Enalaprilat and enalapril diketopiperazine—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS, pH 2.2 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: the peak area of enalaprilat, having the relative retention time of about 0.5 with respect to enalapril obtained from the sample solution, is not larger than 2 times the peak area of enalapril from the standard solution. Also, the peak area of enalapril diketopiperazine, having the relative retention time of about 1.5 is not larger than the peak area of enalapril from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.2 to make exactly 10 mL. Confirm that the peak area of enalapril obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 50 μL of the standard solution.

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

System repeatability: When the test is repeated 6 times

with 50 μL of the standard solution under the above conditions, the relative standard deviation of the peak area of enalapril 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.

Take 1 tablet of Enalapril Maleate Tablets, add $V/2$ mL of sodium dihydrogen phosphate TS, pH 2.2, treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and add sodium dihydrogen phosphate TS, pH 2.2 to make exactly V mL so that 1 mL of the solution contains about 0.1 mg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$). Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V/200)$

W_S: Amount (mg) of Enalapril Maleate 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 rates in 15 minutes of a 2.5- and 5-mg tablet of Enalapril Maleate Tablets and in 30 minutes of a 10-mg tablet of Enalapril Maleate Tablets are not less than 85%, respectively.

Start the test with 1 tablet of Enalapril 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 water to make exactly V' mL so that each mL contains about 2.8 μg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of Enalapril Maleate Reference Standard, previously dried in vacuum at 60°C for 2 hours, and dissolve in water to make exactly 500 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 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 enalapril peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S: Amount (mg) of Enalapril Maleate Reference Standard

C: Labeled amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$) in 1 tablet

Operating conditions—

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

Mobile phase: Dissolve 1.88 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.2 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 250 mL of acetonitrile.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above conditions, the number of theoretical plates and the symmetry factor of the peak of enalapril are not less than 300 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 conditions, the relative standard deviation of the peak area of enalapril is not more than 2.0%.

Assay Weigh accurately not less than 20 Enalapril Maleate Tablets, and powder. Weigh accurately a portion of the powder equivalent to about 10 mg of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$), add 50 mL of sodium dihydrogen phosphate TS, pH 2.2, treat with ultrasonic waves for 15 minutes, shake for 30 minutes, and then add sodium dihydrogen phosphate TS, pH 2.2 to make exactly 100 mL. Treat this solution with ultrasonic waves for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 20 mg of Enalapril Maleate Reference Standard, previously dried in vacuum at 60°C for 2 hours, dissolve in sodium dihydrogen phosphate TS, pH 2.2 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 enalapril peak areas, A_T and A_S, of both solutions.

Amount (mg) of enalapril maleate ($C_{20}H_{28}N_2O_5.C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (1/2)$

W_S: Amount (mg) of Enalapril Maleate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

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

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

Mobile phase: A mixture of sodium dihydrogen phosphate TS, pH 2.2 and acetonitrile (3:1).

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

System suitability—

System performance: Heat to fusion about 20 mg of enalapril maleate. After cooling, add 50 mL of acetonitrile, and treat with ultrasonic waves to dissolve. To 1 mL of this solution, add the standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 50 μL of the solution for system

suitability test under the above conditions, enalapril and enalapril diketopiperazine, which has a relative retention time of 1.5 to enalapril, 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 50 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of enalapril is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Injection

エフェドリン塩酸塩注射液

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 Membrane filtration method: it meets the requirement.

Ephedrine Hydrochloride Tablets

エフェドリン塩酸塩錠

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 Ephedrine Hydrochloride Tablets add V mL of water so that each mL contains 0.25 mg of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$), then add exactly $V/4$ mL of the internal standard solution, disperse the tablet into small particles using ultrasonic waves, then stir for a further 10 minutes in the same way. Shake this solution for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water 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. Then, proceed as directed in the Assay.

Amount (mg) of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$) $= W_S \times (Q_T/Q_S) \times (V/100)$

W_S: Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of etilefrine hydrochloride (1 in 2000).

Add the following:

Erythromycin Enteric-Coated Tablets

エリスロマイシン腸溶錠

Erythromycin Enteric-Coated Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

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

Identification To a quantity of powdered Erythromycin Enteric-Coated Tablets, equivalent to 10 mg (potency) of Erythromycin according to the labeled amount, add 1 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of Erythromycin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Erythromycin.

Loss on drying ⟨*2.41*⟩ Not more than 10.0% (0.2 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Disintegration ⟨*6.09*⟩ 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 Erythromycin.

(ii) Sample solutions—Weigh accurately the mass of not less than 20 Erythromycin Enteric-Coated Tablets, and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency) of Erythromycin, add 25 mL of methanol, shake vigorously, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 100 mL, and filter. Take exactly an appropriate volume of the filtrate, add 0.1 mol/L phosphate buffer solution, pH 8.0, to prepare solutions containing 20 μg (potency) and 5 μg (potency) per mL, and use these solutions as the high and low concentration sample solutions, respectively.

Containers and storage Containers—Well-closed containers.

Add the following:

Etizolam Fine Granules

エチゾラム細粒

Etizolam Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$: 342.85).

Method of preparation Prepare fine particles as directed under Powders, with Etizolam.

Identification **(1)** To a quantity of powdered Etizolam Fine Granules, equivalent to 5 mg of Etizolam according to the labeled amount, add 10 mL of methanol, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness on a water bath, cool, and then dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescent when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Fine Granules, equivalent to 1 mg of Etizolam according to the labeled amount, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm, when perform the measurement within 10 minutes.

Uniformity of dosage units *<6.02>* The granules in single-unit container meet 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 30 minutes of Etizolam Fine Granules is not less than 75%.

Start the test with an accurately weighed amount of Etizolam Fine Granules, equivalent to about 1 mg of etizolam ($C_{17}H_{15}ClN_4S$) 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 filtrate, pipet the subsequent 2 mL, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, 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 enalapril peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$)

$$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times (18/5)$$

W_S: Amount (mg) of etizolam for assay

W_T: Amount (g) of sample

C: Labeled amount (mg) of etizolam ($C_{17}H_{15}ClN_4S$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 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 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 7 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 etizolam 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 etizolam is not more than 2.0%.

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

Assay Weigh accurately an amount of Etizolam Fine Granules, equivalent to about 4 mg of etizolam ($C_{17}H_{15}ClN_4S$), add 30 mL of water, and stir. Add 60 mL of methanol, stir for 20 minutes, add methanol to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add diluted methanol (7 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add diluted methanol (7 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (7 in 10) to make 25 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 etizolam to that of the internal standard.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = W_S \times (Q_T/Q_S) \times (1/25)$$

W_S: Amount (mg) of etizolam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

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

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

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

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

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 etizolam 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 ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Etizolam Tablets

エチゾラム錠

Etizolam Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of etizolam ($C_{17}H_{15}ClN_4S$: 342.85).

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

Identification **(1)** To a quantity of powdered Etizolam Tablets, equivalent to 5 mg of Etizolam according to the labeled amount, add 10 mL of methanol, shake, and filter. Evaporate the filtrate to dryness on a water bath, cool, and then dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescence when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Tablets, equivalent to 1 mg of Etizolam according to the labeled amount, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm when perform the measurement within 10 minutes.

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 Etizolam Tablets, add 2.5 mL of water, and stir until it disintegrates. Add 20 mL of methanol, stir for 20 minutes, add methanol to make exactly 25 mL, and centrifuge. Pipet V mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL so that each mL contains about 8 μg of etizolam ($C_{17}H_{15}ClN_4S$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = W_S \times (Q_T/Q_S) \times (1/V) \times (1/20)$$

W_S: Amount (mg) of etizolam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 50,000).

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 Etizolam Tablets is not less than 70%.

Start the test with 1 tablet of Etizolam 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 etizolam ($C_{17}H_{15}ClN_4S$) according to the labeled amount. Pipet 2 mL of the solution, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, 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 of etizolam, A_T and A_S, of both solutions.

$$\text{Dissolution rate (\%) with respect to the labeled amount of etizolam } (C_{17}H_{15}ClN_4S) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/5)$$

W_S: Amount (mg) of etizolam for assay

C: Labeled amount (mg) of etizolam ($C_{17}H_{15}ClN_4S$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 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 and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 7 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 etizolam 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 etizolam is not more than 2.0%.

Assay To 20 Etizolam Tablets add 50 mL of water, and stir until they disintegrate. Add 400 mL of methanol, stir for 20 minutes, add methanol to make exactly 500 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 0.2 mg of etizolam ($C_{17}H_{15}ClN_4S$), add exactly 10 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 100 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (9 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add diluted methanol (9 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 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 etizolam to that of the internal standard.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = W_S \times (Q_T/Q_S) \times (1/500)$$

W_S: Amount (mg) of etizolam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 50,000).

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 particle diameter).

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

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

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

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 etizolam 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 ratio of the peak area of etizolam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Famotidine for Injection

注射用ファモチジン

Add the following next to Bacterial endotoxins:

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.

Faropenem Sodium Hydrate

ファロペネムナトリウム水和物

Change the Purity to read:

Purity

(1) Heavy metals <*1.07*>—Proceed with 2.0 g of Faropenem Sodium 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—Dissolve a quantity of Faropenem Sodium Hydrate equivalent to 0.10 g (potency) in 200 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 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 by the automatic integration method: the peak area of the epimer, having the relative retention time of about 1.1 with respect to faropenem, obtained from the sample solution is not larger than 3/10 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem

from the sample solution is not larger than 1/2 times the peak area of faropenem from the standard solution.

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: 240 nm).

Time span of measurement: About 6 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, *m*-hydroxyacetophenone and faropenem 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 faropenem is not more than 2.0%.

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 Identification:

Purity Related substances—Powder Faropenem Sodium for Syrup, if necessary. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 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: the area of the peak of cleaved derivative, having the relative retention time of about 0.71 with respect to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem from the sample solution is not larger than 2 times the peak area of faropenem from the standard solution. For these calculations use the area of the peak of cleaved derivative, having the relative retention time of 0.71, after multiplying by the relative response factor 0.37.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra *n*-butylammonium bromide in water to make 1000 mL.

Mobile phase B: A mixture of the mobile phase A and acetonitrile (1:1).

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 – 54	84→30	16→70

Flow rate: About 1.5 mL per minute

Time span of measurement: About 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, *m*-hydroxyacetophenone and faropenem are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

Faropenem Sodium Tablets

ファロペネムナトリウム錠

Add the following next to Identification:

Purity Related substances—Powder not less than 5 Faropenem Sodium Tablets. To a part of the powder, equivalent to about 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add about 10 mL of water, shake well, then add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 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: the area of the peak of cleaved derivative, having the relative retention time of about 0.71 with respect to faropenem, obtained from the sample solution is not larger than 1.5 times the peak area of faropenem from the standard solution, and the total area of the peaks other than the peak of faropenem from the sample solution is not larger than 2.5 times the peak area of faropenem from the standard solution. For these calculation, use the area of the peak of cleaved derivative, having the relative retention time of 0.71, after multiplying by the relative response factor 0.37.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase A: Dissolve 6.12 g of potassium dihydrogen phosphate, 1.79 g of disodium hydrogen phosphate dodecahydrate and 1.61 g of tetra *n*-butylammonium bromide in water to make 1000 mL.

Mobile phase B: A mixture of the mobile phase A and acetonitrile (1:1).

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 – 54	84→30	16→70

Flow rate: About 1.5 mL per minute

Time span of measurement: About 2.5 times as long as the retention time of faropenem, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of faropenem obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution obtained in the Assay under the above operating conditions, *m*-hydroxyacetophenone and faropenem are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of faropenem is not more than 3.0%.

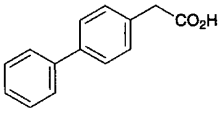
Add the following next to Uniformity of dosage units:

Disintegration ⟨*6.09*⟩ It meets the requirement.

Add the following:

Felbinac

フェルビナク



$C_{14}H_{12}O_2$: 212.24

Biphenyl-4-ylacetic acid [*5728-52-9*]

Felbinac, when dried, contains not less than 98.5% and not more than 101.0% of $C_{14}H_{12}O_2$.

Description Felbinac occurs as white to pale yellowish white crystals or crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Felbinac in methanol (95) (1 in 200,000) 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 Felbinac 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.

Melting point ⟨*2.60*⟩ 163 – 166°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Felbinac in 40 mL of acetone, 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 by combining 0.3 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.011%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Felbinac 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 Felbinac in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of this solution, and add acetone to make exactly 100 mL. Pipet 5 mL of this solution, add acetone 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 heptane, acetone, and acetic acid (100) (50:25:1) to a distance of about 12 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution are not more in-

tense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Felbinac, previously dried, dissolve in 50 mL of methanol, add 15 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
= 21.22 mg of $C_{14}H_{12}O_2$

Containers and storage Containers—Tight containers.

Folic Acid Injection

葉酸注射液

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 Membrane filtration method: it meets the requirement.

Folic Acid Tablets

葉酸錠

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 Folic Acid Tablets add 50 mL of dilute sodium hydroxide TS, shake frequently, and filter. Wash the residue with dilute sodium hydroxide TS, combine the filtrate and the washings, then add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 30 mL of this solution, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, allow to stand for 20 minutes, and filter the solution through a dried filter paper. 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 15 μg of folic acid ($C_{19}H_{19}N_7O_6$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid Reference Standard (separately determine the water ⟨*2.48*⟩ in the same manner as Folic Acid), and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 30 mL of this solutions, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 60 mL of this solution, add 0.5 g of zinc powder, shake frequently, allow to stand for 20 minutes, and filter the solution through a dried filter paper. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of sodium nitrite solution (1 in 1000) to them, mix, and allow to stand for 2 minutes. To these solutions add 1 mL of a solution of ammonium amidosulfate (1 in 200), shake, and allow them to stand for 2 minutes. To these solutions add 1 mL of a solution of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, pipet 30 mL of the sample stock solution, add 20 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet V mL of this solution, and add water to make exactly V' mL so that each mL contains about 15 μg of folic acid ($C_{19}H_{19}N_7O_6$). With exactly 4 mL of this solution perform the same procedure described above for obtaining the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances at 550 nm, A_T, A_S and A_C, of the solutions obtained from the sample solution and standard solution, and the blank solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a control solution obtained with 4 mL of water in the same manner as described above.

$$\text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) = W_S \times \{(A_T - A_C)/A_S\} \times (V'/V) \times (1/10)$$

W_S: Amount (mg) of Folic Acid Reference Standard, calculated on the anhydrous basis

Delete the following two Monographs:

Fosfestrol

ホスフェストロール

Fosfestrol Tablets

ホスフェストロール錠

Fructose Injection

果糖注射液

Delete the Pyrogen and add the following next to Residue on ignition:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.5 EU/mL.

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.

Gabexate Mesilate

ガベキサートメシル酸塩

Change the Identification (4) to read:

Identification (4) A 0.1 g portion of Gabexate Mesilate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for mesilate.

Glucose Injection

ブドウ糖注射液

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

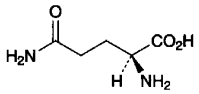
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

L-Glutamine

L-グルタミン



$C_5H_{10}N_2O_3$: 146.14
(2*S*)-2,5-Diamino-5-oxopentanoic acid [*56-85-9*]

L-Glutamine, when dried, contains not less than 99.0% and not more than 101.0% of $C_5H_{10}N_2O_3$.

Description L-Glutamine occurs as white crystals or a crystalline powder. It has a slight characteristic taste.

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

Identification Determine the infrared absorption spectrum of L-Glutamine 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*⟩ $[\alpha]_D^{20}$: +6.3 – +7.3° Weigh accurately about 2 g of L-Glutamine, previously dried, add 45 mL of water, warm to 40°C to dissolve, and after cooling, add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell, within 60 minutes.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.0 g of L-Glutamine in 50 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 0.5 g of L-Glutamine in 20 mL of water is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Glutamine. 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-Glutamine. 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 0.10 g of L-Glutamine, using the distillation under reduced pressure. Prepare the control solution with 10.0 mL of Standard Ammonium Solution. The temperature of the water bath is 45°C (not more than 0.1%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Glutamine 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-Glutamine 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—Dissolve 0.10 g of L-Glutamine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under 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. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of L-Glutamine, 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
= 14.61 mg of $C_5H_{10}N_2O_3$

Containers and storage Containers—Tight containers.

Add the following:

Griseofulvin Tablets

グリセオフルビン錠

Griseofulvin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of griseofulvin ($C_{17}H_{17}ClO_6$: 352.77).

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

Identification To a quantity of powdered Griseofulvin Tablets, equivalent to 15 mg (potency) of Griseofulvin according to the labeled amount, add 100 mL of ethanol (95), shake vigorously, and filter. To 1 mL of the filtrate add ethanol (95) to make 10 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 234 nm and 238 nm, between 290 nm and 294 nm, and between 323 nm and 328 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 1 tablet of Griseofulvin Tablets, add $V/5$ mL of water, treat with ultrasonic waves to disintegrate the tablet, add *N*,*N*-dimethylformamide to make $5V/8$ mL, shake vigorously for 20 minutes, add *N*,*N*-dimethylformamide to make exactly V mL so that each mL contains 1.25 mg (potency) of Griseofulvin, and centrifuge. Pipet 8 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make 100 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed under the Assay.

Amount [mg (potency)] of griseofulvin ($C_{17}H_{17}ClO_6$) $= W_S \times (Q_T/Q_S) \times (V/32)$

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

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 2000).

Disintegration ⟨*6.09*⟩ It meets the requirement.

Assay Weigh accurately not less than 20 Griseofulvin Tablets, and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g (potency) of Griseofulvin, add 50 mL of water, and treat with ultrasonic waves. Add 100 mL of *N*,*N*-dimethylformamide, shake vigorously for 20 minutes, and add *N*,*N*-dimethylformamide to make exactly 250 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add water to make 100 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Griseofulvin Reference Standard, equivalent to about 40 mg (potency), and dissolve in *N*,*N*-dimethylformamide to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, add water 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 griseofulvin to that of the internal standard.

Amount [mg (potency)] of griseofulvin ($C_{17}H_{17}ClO_6$) $= W_S \times (Q_T/Q_S) \times (25/2)$

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

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 2000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Griseofulvin.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, griseofulvin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

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

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride Tablets

ヒドララジン塩酸塩錠

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 Hydralazine Hydrochloride Tablets add 25 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and centrifuge. Pipet V mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 10 μg of hydralazine hydrochloride ($C_8H_8N_4$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.1 mol/L hydrochloric acid

TS to make exactly 50 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 at 260 nm, A_{T1} and A_{S1}, and at 350 nm, A_{T2} and A_{S2}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of hydralazine hydrochloride ($C_8H_8N_4.HCl$) $= W_S \times \{(A_{T1} - A_{T2})/(A_{S1} - A_{S2})\} \times (V'/V) \times (1/50)$

W_S: Amount (mg) of hydralazine hydrochloride for assay

Change to read:

Hypromellose Phthalate

ヒプロメロースフタル酸エステル

[*9050-31-1*]

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

Hypromellose Phthalate is a monophthalic acid ester of hypromellose.

It contains methoxy group ($-OCH_3$: 31.03), hydroxypropoxy group ($-OCH_2CHOHCH_3$: 75.09), and carboxybenzoyl group ($-COC_6H_4COOH$: 149.12).

It contains not less than 21.0% and not more than 35.0% of carboxybenzoyl group, calculated on the anhydrous basis.

◆Its substitution type and its viscosity in millipascal second (mPa·s) are shown on the label.

Substitution	Carboxybenzoyl group (%)	
Type	Min.	Max.
200731	27.0	35.0
220824	21.0	27.0

◆

◆**Description** Hypromellose Phthalate occurs as white powder or granules.

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

It becomes a viscous liquid when a mixture of methanol and dichloromethane (1:1) or a mixture of ethanol (99.5) and acetone (1:1) is added.

It dissolves in sodium hydroxide TS.◆

◆**Identification** Determine the infrared absorption spectrum of Hypromellose Phthalate 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.◆

Viscosity <*2.53*> To 10 g of Hypromellose Phthalate, previously dried at 105°C for 1 hour, add 90 g of a mixture of methanol and dichloromethane in equal mass ratio, and stir to dissolve. Determine the viscosity at 20 ± 0.1°C as directed in Method 1 under Viscosity Determination: the viscosity is not less than 80% and not more than 120% of the labeled unit.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Hypromellose Phthalate in 40 mL of 0.2 mol/L sodium hydroxide VS, add 1 drop of phenolphthalein TS, and add dilute nitric acid dropwise with vigorous stirring until the red color is discharged. Further add 20 mL of dilute nitric acid with stirring. Heat on a water bath with stirring until the gelatinous precipitate formed turns to granular particles. After cooling, centrifuge, and take off the supernatant liquid. Wash the precipitate with three 20-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, add water to make 200 mL, and filter. Perform the test with 50 mL of the filtrate. Control solution: To 0.50 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of 0.2 mol/L sodium hydroxide VS and 7 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.07%).

◆**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Hypromellose Phthalate 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) Phthalic acid—Weigh accurately about 0.2 g of Hypromellose Phthalate, add about 50 mL of acetonitrile to dissolve partially with the aid of ultrasonic waves, add 10 mL of water, and dissolve further with the ultrasonic waves. After cooling, add acetonitrile to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of phthalic acid, dissolve in about 125 mL of acetonitrile by mixing, add 25 mL of water, then add acetonitrile to make exactly 250 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 phthalic acid, A_T and A_S, of both solutions: amount of phthalic acid ($C_8H_6O_4$: 166.13) is not more than 1.0%.

Amount (%) of phthalic acid $= (W_S/W_T) \times (A_T/A_S) \times 40$

W_S: Amount (mg) of phthalic acid

W_T: Amount (mg) of sample, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of 0.1% trifluoroacetic acid and acetonitrile (9:1).

Flow rate: About 2.0 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 phthalic acid are not less than 2500 and not more than 1.5, respectively.◆

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

Water ⟨*2.48*⟩ Not more than 5.0% (1 g, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for Karl Fischer method).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 1 g of Hypromellose Phthalate, dissolve in 50 mL of a mixture of ethanol (95), acetone and water (2:2:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Amount (\%) of carboxybenzoyl group } (C_8H_5O_3) = \{(0.01 \times 149.1 \times V)/W\} - \{(2 \times 149.1 \times P)/166.1\}$$

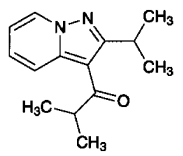
P: Amount (%) of phthalic acid obtained in the Purity (3)
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
W: Amount (g) of sample, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Add the following:

Ibudilast

イブジラスト



$C_{14}H_{18}N_2O$: 230.31
1-[2-(1-Methylethyl)pyrazolo[1,5-*a*]pyridin-3-yl]-2-methylpropan-1-one [*50847-11-5*]

Ibudilast, when dried, contains not less than 98.5% and not more than 101.0% of $C_{14}H_{18}N_2O$.

Description Ibudilast occurs as a white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Ibudilast in methanol (1 in 250,000) 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 Ibudilast 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*⟩ 54 – 58°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ibudilast according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Ibudilast in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 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, and determine each peak area by the automatic integration method: the peak area other than ibudilast obtained from the sample solution is not larger than the peak area of ibudilast from the standard solution, and the total area of the peaks other than ibudilast from the sample solution is not larger than 3 times the peak area of ibudilast from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 2.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of hexane and ethyl acetate (50:1)

Flow rate: Adjust the flow rate so that the retention time of ibudilast is about 9 minutes.

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

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ibudilast obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: To 5 mL of the sample solution add the mobile phase to make 50 mL. To 2 mL of this solution add the mobile phase to make 20 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 ibudilast are not less than 3500 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 operat-

ing conditions, the relative standard deviation of the peak area of ibudilast is not more than 3.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, in vacuum, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ibudilast, previously dried, dissolve in 50 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
= 23.03 mg of $C_{14}H_{18}N_2O$

Containers and storage Containers—Tight containers.

Idoxuridine Ophthalmic Solution

イドクスウリジン点眼液

Add the following next to Purity:

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.

Imipramine Hydrochloride Tablets

イミプラミン塩酸塩錠

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 Imipramine Hydrochloride Tablets add exactly 40 mL of 0.01 mol/L hydrochloric acid TS, disperse the tablet into a small particles using ultrasonic waves, then shake well. Centrifuge the solution, pipet V mL of the supernatant liquid, add water to make exactly V' mL so that each mL contains about 20 μg of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances at 251 nm, A_{T1} and A_{S1}, and at 330 nm, A_{T2} and A_{S2}, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2$.HCl)
$= W_S \times \{(A_{T1} - A_{T2})/(A_{S1} - A_{S2})\} \times (V'/V) \times (4/125)$

W_S: Amount (mg) of Imipramine Hydrochloride Reference Standard

Indometacin Capsules

インドメタシンカプセル

Add the following next to Purity:

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 content of 1 capsule of Indometacin Capsules, and dissolve in methanol to make exactly V mL so that each mL contains about 1 mg of indometacin ($C_{19}H_{16}ClNO_4$). Filter the solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Indometacin Reference Standard, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of indometacin ($C_{19}H_{16}ClNO_4$)
$= W_S \times (Q_T/Q_S) \times (V/25)$

W_S: Amount (mg) of Indometacin Reference Standard

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

Isotonic Sodium Chloride Solution

生理食塩液

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

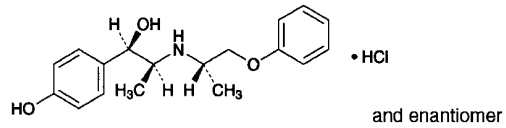
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Isoxsuprine Hydrochloride

イソクスプリン塩酸塩



$C_{18}H_{23}NO_3$.HCl: 337.84
(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-{[(2*SR*)-1-phenoxypropan-2-yl]amino}propan-1-ol monohydrochloride [*579-56-6*]

Isoxsuprine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{18}H_{23}NO_3$.HCl.

Description Isoxsuprine Hydrochloride occurs as a white, powder or crystalline powder.

It is soluble in formic acid and in methanol, and slightly soluble in water and in ethanol (99.5).

Melting point: about 204°C (with decomposition).

A solution of Isoxsuprine Hydrochloride in methanol (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Isoxsuprine Hydrochloride (1 in 20,000) 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 Isoxsuprine 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 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 0.5 g of Isoxsuprine Hydrochloride in 50 mL of water by warming, and cool: the pH of the solution is between 4.5 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.1 g of Isoxsuprine Hydrochloride in 10 mL of water, warm if necessary, and cool: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isoxsuprine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Isoxsuprine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 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: each peak area other than isoxsuprine obtained from the sample solution is not larger than the peak area of isoxsuprine from the standard solution, and the total area of the peaks other than the peak of isoxsuprine from the sample solution is not larger than 2 times the peak area of isoxsuprine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 269 nm).

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

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

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 770 mL of this solution add 230 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of isoxsuprine is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of isoxsuprine, 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 isoxsuprine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To 1 mL of the sample solution add 2.5 mL of a solution of methyl parahydroxybenzoate (1 in 25,000) and the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and isoxsuprine 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 isoxsuprine is not more than 2.5%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Isoxsuprine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 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
= 33.78 mg of $C_{18}H_{23}NO_3$.HCl

Containers and storage Containers—Well-closed containers.

Add the following:

Isoxsuprine Hydrochloride Tablets

イソクスプリン塩酸塩錠

Isoxsuprine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl: 337.84).

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

Identification To a quantity of powdered Isoxsuprine Hydrochloride Tablets, equivalent to 10 mg of Isoxsuprine Hydrochloride according to the labeled amount, add 150 mL of water, shake, and then add water to make 200 mL. Centrifuge this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 267 nm and 271 nm, and between 272 nm and 276 nm.

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

Add methanol to 1 tablet of Isoxsuprine Hydrochloride Tablets, and shake to disintegrate. Add methanol to make exactly V mL so that each mL contains about 0.4 mg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl)
$= W_S \times (A_T/A_S) \times V \times (1/100)$

W_S: Amount (mg) of isoxsuprine hydrochloride 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 15 minutes of Isoxsuprine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Isoxsuprine 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 11 μg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of isoxsuprine hydrochloride for assay, previously dried at 105°C for 1 hour, 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 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 isoxsuprine peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of isoxsuprine hydrochloride for assay
C: Labeled amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.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 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isoxsuprine 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 isoxsuprine is not more than 2.0%.

Assay Weigh accurately not less than 20 Isoxsuprine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder equivalent to about 40 mg of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl), add 60 mL of methanol, shake for 20 minutes, and then add methanol to make exactly 100 mL. Centrifuge a portion of this solution, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of isoxsuprine hydrochloride for assay, previously dried at 105°C for 1 hour, and dissolve in methanol to make exactly 100 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 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 isoxsuprine in each solution.

Amount (mg) of isoxsuprine hydrochloride ($C_{18}H_{23}NO_3$.HCl)
$= W_S \times (A_T/A_S)$

W_S: Amount (mg) of isoxsuprine hydrochloride for assay

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 40°C.

Mobile phase: Dissolve 4.3 g of diammonium hydrogen phosphate and 3.2 g of sodium 1-pentane sulfonate in water to make 1000 mL, and adjust to pH 2.5 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 isoxsuprine is about 9 minutes.

System suitability—

System performance: To exactly 1 mL of the standard solution add the mobile phase to make exactly 50 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 isoxsuprine are not less than 2000 and not more than 2.0, respectively.

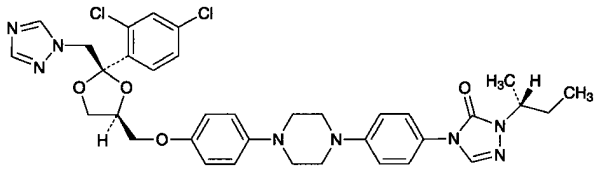
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoxsuprine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

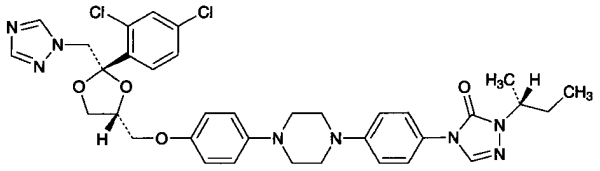
Add the following:

Itraconazole

イトラコナゾール



and enantiomer



and enantiomer

$C_{35}H_{38}Cl_2N_8O_4$: 705.63

4-(4-{4-[4-({(2*RS*,4*SR*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one

4-(4-{4-[4-({(2*SR*,4*RS*)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy)phenyl]piperazin-1-yl}phenyl)-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one

[*84625-61-6*]

Itraconazole contains not less than 98.5% and not more than 101.0% of $C_{35}H_{38}Cl_2N_8O_4$, calculated on the dried basis.

Description Itraconazole occurs as a white powder.

It is soluble in *N*,*N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water and in 2-propanol.

A solution of Itraconazole in *N*,*N*-dimethylformamide (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Itraconazole in 2-propanol (1 in 100,000) 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 Itraconazole, 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 Itraconazole as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 166 – 170°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Itraconazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Itraconazole in 10 mL of a mixture of methanol and tetrahydrofuran (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of each peak other than itraconazole obtained from the sample solution is not larger than the peak area of itraconazole from the standard solution. Furthermore, the total area of the peaks other than itraconazole from the sample solution is not larger than 2.5 times the peak area of itraconazole 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 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of Tetrabutylammonium hydrogensulfate (17 in 625).

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mix-

ing 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 – 20	80→50	20→50
20 – 25	50	50

Flow rate: 1.5 mL per minute.

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of methanol and tetrahydrofuran (1:1) to make exactly 10 mL. Confirm that the peak area of itraconazole obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 1 mg of Itraconazole and 1 mg of miconazole nitrate in 20 mL of the mixture of methanol and tetrahydrofuran (1:1). When the procedure is run with 10 μL of this solution under the above operating conditions, miconazole and itraconazole 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 itraconazole is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Itraconazole, 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
= 35.28 mg of $C_{35}H_{38}Cl_2N_8O_4$

Containers and storage Containers—Tight containers.

Add the following:

Josamycin Tablets

ジョサマイシン錠

Josamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of josamycin ($C_{42}H_{69}NO_{15}$: 827.99).

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

Identification To a quantity of powdered Josamycin Tablets, equivalent to 10 mg (potency) of Josamycin according to the labeled amount, add 100 mL of methanol, shake vigorously, and centrifuge. To 5 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 229 nm and 233 nm.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, in vacuum, 60°C, 3 hours).

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 Josamycin Tablets, add 5 mL of water, and shake vigorously to disintegrate the tablet. Add methanol and then use ultrasonic waves to disperse the particles, add methanol to make exactly V mL so that each mL contains about 2 mg (potency) of Josamycin, and centrifuge. Pipet 3 mL of the supernatant liquid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately weigh about 50 mg (potency) of Josamycin Reference Standard, dissolve in 5 mL of water and methanol to make exactly 25 mL. Pipet 3 mL of this solution, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol 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 the standard solution at 231 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩. However, $\overline{X}$ in the formula for calculation of acceptance value is the result of the assay.

Amount [mg (potency)] of josamycin ($C_{42}H_{69}NO_{15}$)
$= W_S \times (A_T/A_S) \times (V/25)$

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

Disintegration ⟨*6.09*⟩ 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 Josamycin.

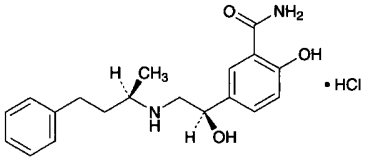
(ii) Sample solutions—Weigh accurately the mass of not less than 20 Josamycin Tablets and pulverize into a powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of Josamycin, add 50 mL of methanol, shake vigorously, and add water to make exactly 1000 mL. Take exactly an appropriate amount of this solution, add water to prepare solutions containing 30 μg (potency) and 7.5 μg (potency) per mL, and use these solutions as the high and low concentration sample solutions, respectively.

Containers and storage Containers—Tight containers.

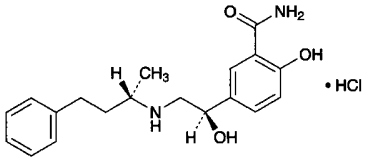
Add the following:

Labetalol Hydrochloride

ラベタロール塩酸塩



and enantiomer



and enantiomer

$C_{19}H_{24}N_2O_3.HCl$: 364.87

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*RS*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

2-Hydroxy-5-{(1*RS*)-1-hydroxy-2-[(1*SR*)-1-methyl-3-phenylpropylamino]ethyl}benzamide monohydrochloride

[*32780-64-6*]

Labetalol Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{19}H_{24}N_2O_3.HCl$.

Description Labetalol Hydrochloride occurs as a white crystalline powder.

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

It dissolves in 0.05 mol/L sulfuric acid TS.

Melting point: about 181°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Labetalol Hydrochloride in 0.05 mol/L sulfuric acid TS (1 in 20,000) 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 Labetalol Hydrochloride as directed in the potassium chloride 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.

(3) A solution of Labetalol Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> The pH of a solution prepared by dissolving 0.5 g of Labetalol Hydrochloride in 50 mL of water is between 4.0 and 5.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Labetalol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.8 g of Labetalol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under 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, 2-propanol, water, and ammonia solution (28) (25:15:8:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution do not exceed 2 in number and are not more intense than the spot obtained from the standard solution.

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

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

Isomer ratio Dissolve 5 mg of Labetalol Hydrochloride in 0.7 mL of a solution of *n*-butylboronic acid in anhydrous pyridine (3 in 250), 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 areas of two adjacent peaks, A_a and A_b, where A_a is the peak area of the shorter retention time and A_b is the peak area of the longer retention time, using the automatic integration method: the ratio $A_b/(A_a+A_b)$ is between 0.45 and 0.55.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 25 m in length, coated inside with methyl silicone polymer for gas chromatography in 5 μm thickness.

Column temperature: A constant temperature of about 290°C.

Injection port temperature: A constant temperature of about 350°C.

Detector temperature: A constant temperature of about 350°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of labetalol is about 9 minutes.

System suitability—

System performance: Proceed with 2 μL of the sample solution under the above conditions: the resolution between the two labetalol peaks is not less than 1.5.

System repeatability: Repeat the test 6 times under the above conditions with 2 μL of the sample solution: the relative standard deviation of the ratio of the peak area of labetalol with the shorter retention time to that of the longer retention time is not more than 2.0%.

Assay Weigh accurately about 0.3 g of Labetalol Hydrochloride, previously dried, 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
= 36.49 mg of $C_{19}H_{24}N_2O_3$.HCl

Containers and storage Containers—Tight containers.

Add the following:

Labetalol Hydrochloride Tablets

ラベタロール塩酸塩錠

Labetalol Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl: 364.87).

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

Identification **(1)** To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 5 mg of Labetalol Hydrochloride according to the labeled amount, add 100 mL of 0.05 mol/L sulfuric acid TS, shake, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 300 nm and 304 nm.

(2) To a quantity of powdered Labetalol Hydrochloride Tablets equivalent to 0.25 g of Labetalol Hydrochloride according to the labeled amount, add 25 mL of methanol, shake vigorously for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of labetalol hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test using 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, 2-propanol, water, and ammonia solution (28) (25:15:8:2) 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 and the spot obtained from the 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 Labetalol Hydrochloride Tablets add 5 mL of 0.5 mol/L sulfuric acid TS and 30 mL of water, shake vigorously for 30 minutes, add water to make exactly 50 mL, and filter. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 40 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS 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 302 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl)
$= W_S \times (A_T/A_S) \times (V/40)$

W_S: Amount (mg) of labetalol hydrochloride 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 Labetalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Labetalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at 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, and add water to make exactly V' mL so that each mL contains about 50 μg of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 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 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 302 nm.

Dissolution rate (%) with respect to the labeled amount of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

W_S: Amount (mg) of labetalol hydrochloride for assay
C: Labeled amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Labetalol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl), add 100 mL of 0.5 mol/L sulfuric acid TS and 600 mL of water, shake vigorously for 30 minutes, add water to make exactly 1000 mL, and filter. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, and add 0.05 mol/L sulfuric acid TS to make exactly 25 mL. Pipet 5 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of labetalol hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this so-

lution, add 0.05 mol/L sulfuric 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 302 nm.

Amount (mg) of labetalol hydrochloride ($C_{19}H_{24}N_2O_3$.HCl) $= W_S \times (A_T/A_S) \times 25$

W_S: Amount (mg) of labetalol hydrochloride for assay

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖

Change the origin/limits of content to read:

Anhydrous Lactose is β-lactose or a mixture of β-lactose and α-lactose.

◆The relative quantities of α-lactose and β-lactose in Anhydrous Lactose is labeled as the isomer ratio.◆

Change the Identification to read:

◆**Identification** Determine the infrared absorption spectrum of Anhydrous Lactose, 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 Anhydrous Lactose Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Levallorphan Tartrate Injection

レバロルファン酒石酸塩注射液

Add the following next to Identification:

Bacterial endotoxins <*4.01*> Less than 150 EU/mg.

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.

Magnesium Sulfate Injection

硫酸マグネシウム注射液

Add the following next to Extractable volume:

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

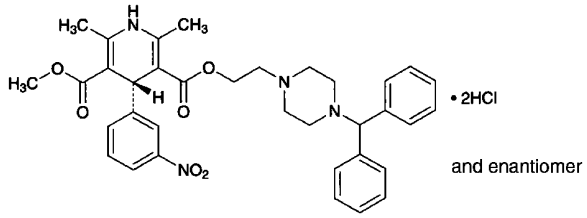
Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Manidipine Hydrochloride

マニジピン塩酸塩



$C_{35}H_{38}N_4O_6$.2HCl: 683.62
3-{2-[4-(Diphenymethyl)piperazin-1-yl]ethyl}
5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-
1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride
[*126229-12-7*]

Manidipine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{35}H_{38}N_4O_6$.2HCl.

Description Manidipine Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

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

A solution of Manidipine Hydrochloride in dimethylsulfoxide (1 in 100) shows no optical rotation.

Manidipine Hydrochloride turns slightly brown-yellowish white on exposure to light.

Melting point: about 207°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Manidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Manidipine 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 Manidipine Hydrochloride as directed in the potassium chloride disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Manidipine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 10 mL of water to 0.1 g of Manidipine Hydrochloride, shake vigorously, and filter. Add 1 drop of

ammonia TS to 3 mL of the filtrate, allow to stand 5 minutes, and filter. The filtrate responds to the Qualitative Tests <*1.09*> (2) for chlorides.

Purity **(1)** Heavy metals <*1.07*>— Proceed with 1.0 g of Manidipine 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).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Manidipine Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 20 mg of Manidipine Hydrochloride in 200 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (1:1) 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 from both solutions by the automatic integration method: the area of the peaks other than manidipine obtained from the sample solution is not larger than 1/5 times the manidipine peak area from the standard solution. Furthermore, the total of the areas of all peaks other than the manidipine peak from the sample solution is not larger than 7/10 times the peak area of manidipine from the standard solution.

Operating conditions—

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

Time span of measurement: About 3.5 times as long as the retention time of manidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of manidipine obtained from 20 μL of this solution is equivalent to 8 to 12% of that from 20 μL of the standard solution.

System performance: Dissolve 50 mg of Manidipine Hydrochloride in a mixture of water and acetonitrile (1:1) to make 50 mL. To 10 mL of this solution add 5 mL of a solution of butyl benzoate in acetonitrile (7 in 5000) and the mixture of water and acetonitrile (1:1) to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, manidipine and butyl 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of manidipine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 1.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Manidipine Hydrochloride, previously dried, and dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride Reference Standard, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) 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 manidipine to that of the internal standard.

$$\text{Amount (mg) of } C_{35}H_{38}N_4O_6.2HCl = W_S \times (Q_T/Q_S) \times 4$$

W_S: Amount (mg) of Manidipine Hydrochloride Reference Standard

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 4.6 with diluted potassium hydroxide TS (1 in 10). To 490 mL of this solution add 510 mL of acetonitrile.

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

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Manidipine Hydrochloride Tablets

マニジピン塩酸塩錠

Manidipine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl: 683.62).

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

Identification To a quantity of powdered Manidipine Hydrochloride Tablets, equivalent to 10 mg of Manidipine Hydrochloride according to the labeled amount, add 5 mL of methanol, shake vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Manidipine Hydrochloride Reference Standard 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 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 and diethylamine (200:1) 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 obtained from the 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.

Conduct this procedure using light-resistant vessels. To 1 tablet of Manidipine Hydrochloride Tablets, add exactly 1 mL of the internal standard solution per 1 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl), disintegrate by adding a mixture of water and acetonitrile (1:1) to make V mL so that each mL contains about 0.1 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl), shake vigorously for 10 minutes, 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. Then, proceed as directed in the Assay.

Amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl)
$= W_S \times (Q_T/Q_S) \times (V/250)$

W_S: Amount (mg) of Manidipine Hydrochloride Reference Standard

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

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 Manidipine Hydrochloride Tablets is not less than 75%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Manidipine 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, and add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl) according to the labeled amount. Pipet 2 mL of this solution, add exactly 2 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Manidipine Hydrochloride Reference Standard, previously dried, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of methanol, 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 manidipine peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S: Amount (mg) of Manidipine Hydrochloride Reference Standard

C: Labeled amount (mg) of manidipine hydrochloride ($C_{35}H_{38}N_4O_6$.2HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 228 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of acetonitrile and a solution of potassium dihydrogen phosphate (681 in 100,000) (3:2).

Flow rate: Adjust the flow rate so that the retention time of manidipine 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 manidipine are not less than 1500 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 manidipine is not more than 2.0%.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately not less than 20 Manidipine Hydrochloride

Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of manidipine hydrochloride ($C_{35}H_{38}N_4O_6.2HCl$), add exactly 10 mL of the internal standard solution, add a mixture of water and acetonitrile (1:1) to make 100 mL, shake vigorously for 10 minutes, 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 about 25 mg of Manidipine Hydrochloride Reference Standard, previously dried, and dissolve in the mixture of water and acetonitrile (1:1) to make 50 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (1:1) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Manidipine Hydrochloride.

$$\text{Amount (mg) of manidipine hydrochloride } (C_{35}H_{38}N_4O_6.2HCl) = W_S \times (Q_T/Q_S) \times (2/5)$$

W_S: Amount (mg) of Manidipine Hydrochloride Reference Standard

Internal standard solution—A solution of butyl benzoate in acetonitrile (7 in 10,000).

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

D-Mannitol Injection

D-マンニトール注射液

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*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Medazepam

メダゼパム

Change the origin/limits of content to read:

Medazepam, when dried, contains not less than 98.5 % and not more than 101.0% of $C_{16}H_{15}ClN_2$.

Change the Description to read:

Description Medazepam occurs as white to light yellow crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (99.5), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually turns yellow on exposure to light.

Change the Identification to read:

Identification (1) Dissolve 10 mg of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) 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 Medazepam 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.

(4) Perform the test with Medazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Mefruside Tablets

メフルシド錠

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 Mefruside Tablets add 40 mL of methanol, disintegrate the tablet using ultrasonic waves with occasional stirring, then further treat with ultrasonic waves for 10 minutes, and add methanol to make exactly V mL of a solution containing about 0.5 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) per mL. Centrifuge the solution, pipet 5 mL of the supernatant liquid, add methanol to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of mefruside } (C_{13}H_{19}ClN_2O_5S_2) = W_S \times (A_T/A_S) \times (V/125)$$

W_S: Amount (mg) of mefruside for assay

Methyldopa Tablets

メチルドパ錠

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 Methyldopa Tablets add 50 mL of 0.05

mol/L sulfuric acid TS, shake for 15 minutes, then add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 5 mg of methyldopa ($C_{10}H_{13}NO_4$), add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa Reference Standard (separately determine the loss on drying <2.41> at 125°C for 2 hours), and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of iron (II) tartrate TS, then add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 520 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of methyldopa } (C_{10}H_{13}NO_4) = W_S \times (A_T/A_S) \times (5/V)$$

W_S: Amount (mg) of Methyldopa Reference Standard, calculated on the dried basis

Add the following:

Minocycline Hydrochloride for Injection

注射用ミノサイクリン塩酸塩

Minocycline Hydrochloride 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 amount of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Method of preparation Prepare as directed under Injections, with Minocycline Hydrochloride.

Description Minocycline Hydrochloride for Injection occurs as a yellow to yellow-brown powder or flakes.

Identification Dissolve 4 mg of Minocycline Hydrochloride for Injection in 250 mL of a solution of hydrochloric acid in methanol (19 in 20,000). Determine the absorption spectrum of this solution 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.

pH <2.54> The pH of a solution, prepared by dissolving an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, in 10 mL of water is 2.0 to 3.5.

Purity Related substances—Conduct this procedure rapidly after the preparation of the sample solution. Take an amount of Minocycline Hydrochloride for Injection, equivalent to 0.1 g (potency) of Minocycline Hydrochloride according to the labeled amount, dissolve in the mobile phase to make 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. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amounts of each peak by the area percentage method: the amount of epiminomycine, having the relative retention time of about 0.83 with respect to minocycline, is not more than 6.0%.

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—

Test for required detectability: Pipet 2 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, 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 from 20 μL of the solution for system suitability test.

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

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

Water <2.48> Weigh accurately the mass of the content of one container of Minocycline Hydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and preform the test with exactly 1 mL of this solution as directed in the Volumetric tiration (back tiration): not more than 3.0%.

Bacterial endotoxins <4.01> Less than 1.25 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 an amount of Minocycline Hydrochloride for Injection, equivalent to about 0.1 g (potency) of Minocycline Hydrochloride, dissolve in the mo-

bile 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, then 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 2.5.

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 minocycline is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Add the following:

Mitomycin C for Injection

注射用マイトマイシン C

Mitomycin C 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 amount of mitomycin C ($C_{15}H_{18}N_4O_5$: 334.33).

Method of preparation Prepare as directed under Injections, with Mitomycin C.

Description Mitomycin C for Injection occurs as a blue-purple powder.

Identification Dissolve an amount of Mitomycin C for Injection, equivalent to 2 mg (potency) of Mitomycin C according to the labeled amount, in 200 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 216 nm and 220 nm, and between 362 nm and 366 nm.

pH <*2.54*> The pH of a solution, prepared by dissolving 0.25 g of Mitomycin C for Injection in 20 mL of water, is 5.5 to 8.5.

Loss on drying <*2.41*> Not more than 1.0% (0.4 g, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 10 EU/mg (potency).

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 container of Mitomycin C for Injection add exactly *V* mL of *N*,*N*-dimethylacetamide so that each mL contains about 0.5 mg (potency) of Mitomycin C, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg (potency) of Mitomycin C Reference Standard, add *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
$= W_S \times (A_T/A_S) \times (V/50)$

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

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 of the contents of not less than 10 containers of Mitomycin C for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Mitomycin C, add exactly 20 mL of *N*,*N*-dimethylacetamide, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount of Mitomycin C Reference Standard, equivalent to about 25 mg (potency), dissolve in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Mitomycin C.

Amount [mg (potency)] of mitomycin C ($C_{15}H_{18}N_4O_5$)
$= W_S \times (A_T/A_S) \times (2/5)$

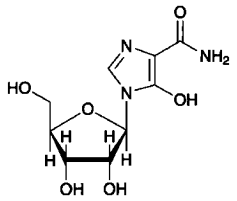
W_S: Amount [mg (potency)] of Mitomycin C Reference Standard

Containers and storage Containers—Hermetic containers.

Add the following:

Mizoribine

ミゾリビン



$C_9H_{13}N_3O_6$: 259.22

5-Hydroxy-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide [*50924-49-7*]

Mizoribine contains not less than 98.0% and not more than 102.0% of $C_9H_{13}N_3O_6$, calculated on the anhydrous basis.

Description Mizoribine occurs as a white to yellowish white crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Mizoribine (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mizoribine 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 Mizoribine 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 Mizoribine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -25 – $-27°$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mizoribine 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 0.10 g of Mizoribine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample 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 areas of the peaks other than mizoribine obtained from the sample solution are not larger than the mizoribine peak area from the standard solution.

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: 220 nm).

Time span of measurement: About 3 times as long as the retention time of mizoribine, 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 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% of that from 5 μL of 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 mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine is not more than 2.0%.

Water <*2.48*> Not more than 0.5% (0.5 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.1 g of Mizoribine, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Mizoribine Reference Standard (separately determine the water <*2.48*> using the same manner as Mizoribine), dissolve in the mobile phase 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 of mizoribine, A_T and A_S, of both solutions.

$$\text{Amount (mg) of } C_9H_{13}N_3O_6 = W_S \times (A_T/A_S) \times 10$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

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

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

Mobile phase: Diluted phosphoric acid (1 in 1500).

Flow rate: Adjust the flow rate so that the retention time of mizoribine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Mizoribine Tablets

ミゾリビン錠

Mizoribine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of mizoribine ($C_9H_{13}N_3O_6$: 259.22).

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

Identification To a quantity of powdered Mizoribine Tablets, equivalent to 0.1 g of Mizoribine according to the labeled amount, add 5 mL of water, shake, filter, and use the filtrate as the sample solution. Separately, dissolve 20 mg of Mizoribine Reference Standard in 1 mL of water, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-Layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop the plate with a mixture of methanol, ammonia solution (28) and 1-propanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the principal spot from the sample solution and the spot from the standard solution show a red-brown color and the same *R*f value.

Purity Related substances—To a quantity of powdered Mizoribine Tablets, equivalent to 0.10 g of Mizoribine according to the labeled amount, add 30 mL of the mobile phase, shake, then add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.5 μm and use the filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of the solution, add the mobile phase 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 each solution by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 with respect to mizoribine, obtained from the sample solution is not larger than the peak area of mizoribine from the standard solution, and the area of each peak other than mizoribine from the sample solution is not larger than 2/5 times the peak area of mizoribine from the standard solution.

Operating conditions—

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

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

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of mizoribine obtained from 5 μL of this solution is equivalent to 14 to 26% of that from 5 μL of 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 mizoribine are not less than 10,000 and not more than 1.4, 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 mizoribine 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 Mizoribine Tablets add 50 mL of water, shake until the tablet is disintegrated, and add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5 μg of mizoribine ($C_9H_{13}N_3O_6$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine Reference Standard (separately determine the water <*2.48*> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 279 nm of the sample solution and standad solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount of mizoribine } (C_9H_{13}N_3O_6) = W_S \times (A_T/A_S) \times (V'/V) \times (1/50)$$

W_S: Amount (mg) of Mizoribine Reference Standard, 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 45 minutes of Mizoribine Tablets is not less than 80%.

Start the test with 1 tablet of Mizoribine 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 not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 14 μg of mizoribine ($C_9H_{13}N_3O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mizoribine Reference Standard (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of mizoribine ($C_9H_{13}N_3O_6$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$

W_S: Amount (mg) of Mizoribine Reference Standard, calculated on the anhydrous basis
C: Labeled amount (mg) of mizoribine ($C_9H_{13}N_3O_6$) in 1 tablet

Assay Weigh accurately not less than 20 Mizoribine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of mizoribine ($C_9H_{13}N_3O_6$), add 50 mL of water and shake, then add water to make exactly 100 mL. Filter the solution, discard not less than 10 mL of the first filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Mizoribine Reference Standard (separately determine the water <2.48> in the same manner as Mizoribine), and dissolve in water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 279 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of mizoribine ($C_9H_{13}N_3O_6$) $= W_S \times (A_T/A_S)$

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

Containers and storage Containers—Tight containers.

Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

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 Morphine Hydrochloride Tablets add exactly 1 mL of the internal standard solution per 2 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), disperse the tablet into a small particles using ultrasonic waves, then treat with ultrasonic waves for 15 minutes with occasional stirring, and add water to make V mL so that each mL contains about 0.4 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$). Filter the solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= W_S \times (Q_T/Q_S) \times (V/50) \times 1.1679$

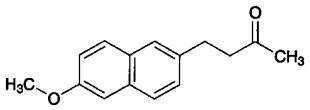
W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Add the following:

Nabumetone

ナブメトン



$C_{15}H_{16}O_2$: 228.29
4-(6-Methoxynaphthalen-2-yl)butan-2-one [*42924-53-8*]

Nabumetone contains not less than 98.0% and not more than 101.0% of $C_{15}H_{16}O_2$, calculated on the anhydrous basis.

Description Nabumetone occurs as white to yellowish white crystals or a crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Nabumetone in methanol (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nabumetone 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 Nabumetone 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 Nabumetone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 79 – 84°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nabumetone 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 20 mg of Nabumetone in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add acetonitrile 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 peak area of the related substance G obtained from the sample solution is not larger than 3/5 times the peak area of nabumetone from the standard solution, and each peak area other than nabumetone and the related substance G from the sample solution is not larger than 1/5 times the peak area of nabumetone from the standard solution. Furthermore, the total area of the peaks other than nabumetone from the sample solution is not larger than 1.6 times the peak area of nabumetone from the standard solution. For these calculations, use each peak area of the related substances A, B, C, D, E, F and G, which are having the relative retention time of about 0.73, 0.85, 0.93, 1.2, 1.9, 2.6 and 2.7 with respect to nabumetone, after multiplying by their relative response factors, 0.12, 0.94, 0.25, 0.42, 1.02, 0.91 and 0.1, respectively.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: A mixture of water and acetic acid (100) (999:1).

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (7:3).

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 – 12	60	40
12 – 28	60→20	40→80

Flow rate: 1.3 mL per minute.

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

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nabumetone obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

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

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nabumetone is not more than 5.0%.

Water ⟨*2.48*⟩ Not more than 0.2% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Nabumetone and Nabumetone Reference Standard (separately determine the water ⟨*2.48*⟩ in the same manner as Nabumetone), dissolve them in acetonitrile to make exactly 20 mL, and use these solutions as the sample solution and the 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 area of nabumetone, A_T and A_S, from each solution.

$$\text{Amount (mg) of } C_{15}H_{16}O_2 = W_S \times (A_T/A_S)$$

W_S: Amount (mg) of Nabumetone 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 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

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

Mobile phase: To 600 mL of a mixture of water and acetic acid (100) (999:1) add 400 mL of a mixture of acetonitrile and tetrahydrofuran (7:3).

Flow rate: Adjust the flow rate so that the retention time of nabumetone 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 number of theoretical plates and the symmetry factor of the peak of nabumetone are not less than 6000 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 nabumetone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Nabumetone Tablets

ナブメトン錠

Nabumetone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nabumetone ($C_{15}H_{16}O_2$: 228.29).

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

Identification To a quantity of powdered Nabumetone Tablets, equivalent to 80 mg of Nabumetone according to the labeled amount, add 50 mL of methanol, shake for 10 minutes and centrifuge the solution. To 1 mL of the supernatant liquid, add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 259 nm and 263 nm, between 268 nm and 272 nm, between 316 nm and 320 nm, and between 330 nm and 334 nm.

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

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80 (dissolving 3 g of polysorbate 80 in water to make 100 mL) as the dissolution medium, the dissolution rate in 60 minutes of Nabumetone Tablets is not less than 70%.

Start the test with 1 tablet of Nabumetone 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 a solution, prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL, to make exactly V' mL so that each mL contains about 89 μg of nabumetone ($C_{15}H_{16}O_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Nabumetone Reference Standard (separately determine the water <*2.48*> in the same manner as Nabumetone), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 331 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared by adding to 20 mL of ethanol (99.5) the dissolution medium to make 50 mL as the blank.

Dissolution rate (%) with respect to the labeled amount of nabumetone ($C_{15}H_{16}O_2$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 360$$

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

C: Labeled amount (mg) of nabumetone ($C_{15}H_{16}O_2$) in 1 tablet

Assay Weigh accurately not less than 20 tablets of Nabumetone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.2 g of nabumetone ($C_{15}H_{16}O_2$), add 10 mL of water and shake, add 40 mL of methanol, shake for 30 minutes, and then add methanol to make exactly 100 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Nabumetone Reference Standard (separately determine the water <*2.48*> in the same manner as Nabumetone), dissolve by adding 50 mL of methanol and exactly 20 mL of the internal standard solution, then add methanol to make 200 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 nabumetone to that of the internal standard.

Amount (mg) of nabumetone ($C_{15}H_{16}O_2$) $= W_S \times (Q_T/Q_S) \times 5$

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

Internal standard solution—Dissolve 0.12 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (550:450:1).

Flow rate: Adjust the flow rate so that the retention time of nabumetone 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, nabumetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.

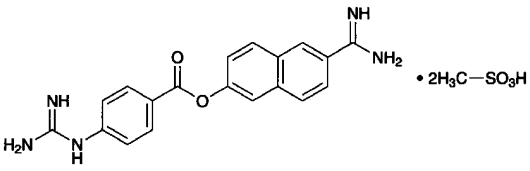
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nabumetone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Nafamostat Mesilate

ナファモスタットメシル酸塩



$C_{19}H_{17}N_5O_2.2CH_4O_3S$: 539.58
6-Amidinonaphthalen-2-yl 4-guanidinobenzoate bis(methanesulfonate) [*82956-11-4*]

Nafamostat Mesilate, when dried, contains not less than 99.0% and not more than 101.0% of $C_{19}H_{17}N_5O_2.2CH_4O_3S$.

Description Nafamostat Mesilate occurs as a white crystalline powder.

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

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 262°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Nafamostat Mesilate in 0.01 mol/L hydrochloric acid TS (1 in 200,000) 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 Nafamostat Mesilate 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 0.1-g portion of Nafamostat Mesilate responds to the Qualitative Tests <*1.09*> (1) for mesilate.

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is between 4.7 and 5.7.

Purity **(1)** Clarity and color of solution—A solution prepared by dissolving 1.0 g of Nafamostat Mesilate in 50 mL of water is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Nafamostat Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Nafamostat Mesilate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mobile phase to make exactly 100 mL. Then pipet 5 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 each solution by the automatic integration method: the area of each peak other than nafamostat obtained from the sample solution is not larger than 1/5 times the peak area of nafamostat from the standard solution. Furthermore, the total area of the peaks other than nafamostat from the sample solution is not larger than the peak area of nafamostat from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

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

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

Mobile phase: Dissolve 6.07 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (3 in 500). To 700 mL of this solution add 300 mL of acetonitrile.

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

Time span of measurement: About 4 times as long as the retention time of nafamostat, 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 50 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of nafamostat obtained from 10 μL of this solution is equivalent to 1.1 to 1.9% of that from 10 μL of the standard solution.

System performance: Dissolve 0.1 g of nafamostat mesilate in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 100 mL. To 5 mL of this solution add 5 mL of a solution of 6-amidino-2-naphthol methanesulfonate in the mobile phase (1 in 20,000). When the procedure is run with 10 μL of this solution under the above operating conditions, 6-amidino-2-naphthol and nafamostat 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 peak area of nafamostat is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.25 g of Nafamostat Mesilate, previously dried, dissolve in 4 mL of formic acid, add 50 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
= 26.98 mg of $C_{19}H_{17}N_5O_2.2CH_4O_3S$

Containers and storage Containers—Tight containers.

Neostigmine Methylsulfate Injection

ネオスチグミンメチル硫酸塩注射液

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 Membrane filtration method: it meets the requirement.

Nicardipine Hydrochloride Injection

ニカルジピン塩酸塩注射液

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 Membrane filtration method: it meets the requirement.

Nicorandil

ニコランジル

Change the Identification to read:

Identification **(1)** Determine the absorption spectrum of a solution of Nicorandil (1 in 50,000) 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 Nicorandil 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.

Nicotinic Acid Injection

ニコチン酸注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 3.0 EU/mg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

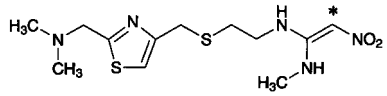
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Nizatidine

ニザチジン



and geometric isomer at C*

$C_{12}H_{21}N_5O_2S_2$: 331.46
(1*EZ*)-*N*-{2-[({2-[(Dimethylamino)methyl]thiazol-4-yl}methyl)sulfanyl]ethyl}-*N′*-methyl-2-nitroethene-1,1-diamine [*76963-41-2*]

Nizatidine, when dried, contains not less than 98.0 % and not more than 101.0% of $C_{12}H_{21}N_5O_2S_2$.

Description Nizatidine occurs as a white to pale yellowish white crystalline powder, and has a characteristic odor.

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

Identification **(1)** Determine the absorption spectrum of a solution of Nizatidine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nizatidine 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 Nizatidine, 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 dried Nizatidine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 130 – 135°C (after drying).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nizatidine according to Method 4, and perform the test using 3 mL of sulfuric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Nizatidine in 10 mL of a mixture of the mobile phase A and mobile phase B (19:6), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of the mobile phase A and mobile phase B (19:6) 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. Determine each peak area from both solutions by the automatic integration method: the area of the peaks other than nizatidine peak obtained from the sample solution is not larger than 1/5 times the nizatidine peak area from the standard solution. Furthermore, the total of the areas of peaks other than the nizatidine peak from the sample solution is not larger than the peak area of nizatidine from the standard solution.

Operating conditions—

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

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

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

Mobile phase A: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100).

Mobile phase B: Methanol.

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 – 3	76	24
3 – 20	76→50	24→50
20 – 45	50	50

Flow rate: 1.0 mL per minute.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of the mobile phase A and mobile phase B (19:6) to make exactly 25 mL. Confirm that the peak area of nizatidine obtained from 50 μL of this solution is equivalent to 15 to 25% of that from 50 μL of 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 nizatidine are not less than 20,000 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 nizatidine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (2 g, 100°C, 1 hour).

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

Assay Weigh accurately about 15 mg each of Nizatidine and Nizatidine Reference Standard, both previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the 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. Determine the peak area of nizatidine, A_T and A_S, from each solution.

$$\text{Amount (mg) of } C_{12}H_{21}N_5O_2S_2 = W_S \times (A_T/A_S)$$

W_S: Amount (mg) of Nizatidine Reference Standard

Operating conditions—

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

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

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

Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nizatidine 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 number of theoretical plates and the symmetry factor of the peak of nizatidine 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 nizatidine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Nizatidine Capsules

ニザチジンカプセル

Nizatidine Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$: 331.46).

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

Identification Take out the contents of Nizatidine Capsules, and powder. To a portion of the powder, equivalent to 50 mg of Nizatidine according to the labeled amount, add 50 mL of methanol, shake well, and filter. Pipet 1 mL of the filtrate, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 239 nm and 244 nm, and between 323 nm and 327 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 from 1 capsule of Nizatidine Capsules, add exactly 50 mL of the mobile phase per 75 mg of Nizatidine, and shake vigorously for 10 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and add the mobile phase to make V' mL so that each mL contains about 0.3 mg of nizatidine ($C_{12}H_{21}N_5O_2S_2$). Use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
$= W_S \times (Q_T/Q_S) \times (V'/V)$

W_S: Amount (mg) of Nizatidine Reference Standard

Internal standard solution—A solution of phenol in the mobile phase (1 in 100).

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

Start the test with 1 capsule of Nizatidine Capsules, 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 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 10 μg of nizatidine ($C_{12}H_{21}N_5O_2S_2$) according to the labeled amount. Use this solution as the sample solution. Separately, weigh accurately about 25 mg of Nizatidine Reference Standard, previously dried at 100°C for 1 hour, 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 the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 314 nm.

Dissolution rate (%) with respect to the labeled amount of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S: Amount (mg) of Nizatidine Reference Standard
C: Labeled amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$) in 1 capsule

Assay Take out the contents of not less than 10 Nizatidine Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of nizatidine ($C_{12}H_{21}N_5O_2S_2$), add exactly 50 mL of the mobile phase, shake vigorously for 10 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, 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 15 mg of Nizatidine Reference Standard, previously dried at 100°C for 1 hour, dissolve in 30 mL of the mobile phase, 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 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 nizatidine to that of the internal standard.

Amount (mg) of nizatidine ($C_{12}H_{21}N_5O_2S_2$)
$= W_S \times (Q_T/Q_S) \times 10$

W_S: Amount (mg) of Nizatidine Reference Standard

Internal standard solution—A solution of phenol in the mobile phase (1 in 100).

Operating conditions—

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

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

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

Mobile phase: Dissolve 5.9 g of ammonium acetate in 760 mL of water, add 1 mL of diethylamine, and adjust to pH 7.5 with acetic acid (100). To this solution add 240 mL of methanol.

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and nizatidine 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 ratio of the peak area of nizatidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Noradrenaline Injection

ノルアドレナリン注射液

Add the following next to Purity:

Bacterial endotoxins ⟨*4.01*⟩ Less than 300 EU/mg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

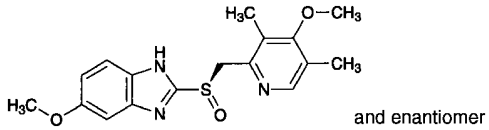
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Omeprazole

オメプラゾール



$C_{17}H_{19}N_3O_3S$: 345.42

(*RS*)-5-Methoxy-2-{[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl}-1*H*-benzoimidazole [*73590-58-6*]

Omeprazole, when dried, contains not less than 99.0 % and not more than 101.0% of $C_{17}H_{19}N_3O_3S$.

Description Omeprazole occurs as a white to yellowish white crystalline powder.

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

A solution of Omeprazole in *N*,*N*-dimethylformamide (1 in 25) shows no optical rotation.

It gradually turns yellowish white on exposure to light.

Melting point: about 150°C (with decomposition).

Identification **(1)** Add phosphate buffer solution, pH 7.4, to 1 mL of a solution of Omeprazole in ethanol (99.5) (1 in 1000) 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 Omeprazole 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)** Clarity and color of solution—Dissolve 0.5 g of Omeprazole in 25 mL of *N*,*N*-dimethylformamide: the solution is clear and colorless or light yellow. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 420 nm is not more than 0.3.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Omeprazole 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 the procedure soon after preparation of the sample solution. Dissolve 50 mg of Omeprazole in 50 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each of the peak areas of the sample solution by the automatic integration method, and calculate the amounts of them by the area percentage method: each of the amount other than omeprazole is not more than 0.1%, and the total amount of the peaks other than omeprazole is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

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

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

Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL. If necessary, adjust the pH to 7.6 with diluted phosphoric acid (1 in 100). Add 11 volumes of acetonitrile to 29 volumes of this solution.

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

Time span of measurement: About 10 times as long as the retention time of omeprazole, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of omeprazole obtained from 10 μL of this solution is equivalent to 15 to 25% of that from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Omeprazole and 25 mg of 1,2-dinitrobenzene in 5 mL of sodium borate solution (19 in 5000) and 95 mL of ethanol (99.5). When the procedure is run with 10 μL of this solution under the above conditions, omeprazole and 1,2-dinitrobenzene 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of omeprazole is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.2% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Omeprazole, previously dried, dissolve in 70 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination using the same method on a solution consisting of 70 mL of *N*,*N*-dimethylformamide and 12 mL of water, and make any necessary correction.

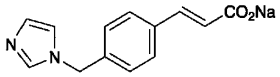
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 34.54 mg of $C_{17}H_{19}N_3O_3S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, in a cold place.

Add the following:

Ozagrel Sodium

オザグレルナトリウム



$C_{13}H_{11}N_2NaO_2$: 250.23
Monosodium (2*E*)-3-[4-(1*H*-imidazol-1-ylmethyl)phenyl]prop-2-enoate [*189224-26-8*]

Ozagrel Sodium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{13}H_{11}N_2NaO_2$.

Description Ozagrel Sodium occurs as white crystals or crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Ozagrel Sodium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ozagrel Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ozagrel Sodium 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 Ozagrel Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ozagrel Sodium (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.5 g of Ozagrel Sodium in 10 mL of water is between 9.5 and 10.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Ozagrel Sodium in 10 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 2.0 g of Ozagrel Sodium in 30 mL of water, add 1 mL of acetic acid (100) and water to make 50 mL, shake, and allow to stand for 30 minutes. Filter the solution, discard the first 5 mL of the filtrate, and to 25 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ozagrel Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 50 mg of Ozagrel Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ 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: each of the amount other than ozagrel is not more than 0.2%, and the total amount other than ozagrel 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: 220 nm).

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

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ozagrel obtained from 5 μL of this solution is equivalent to 15 to 25% of that from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ozagrel are not less than 6000 and not more than 2.0, respectively.

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

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 25 mg each of Ozagrel Sodium and Ozagrel Sodium Reference Standard, both previously dried, and dissolve each in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 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 ozagrel to that of the internal standard.

$$\text{Amount (mg) of } C_{13}H_{11}N_2NaO_2 = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of Ozagrel Sodium Reference Standard

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 a solution of ammonium acetate (3 in 1000) and methanol (4:1).

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

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the internal standard and ozagrel are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of ozagrel is not more than 2.0.

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 ozagrel to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Ozagrel Sodium for Injection

注射用オザグレルナトリウム

Ozagrel Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$: 250.23).

Method of preparation Prepare as directed under Injections, with Ozagrel Sodium.

Description Ozagrel Sodium for Injection occurs as white masses or powder.

Identification Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 40 mg of Ozagrel Sodium according to the labeled amount, in water to make 40 mL. To 1 mL of this solution add water to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 269 nm and 273 nm.

pH Being specified separately.

Purity Related substances—Dissolve an amount of Ozagrel Sodium for Injection, equivalent to 0.20 g of Ozagrel Sodium according to the labeled amount, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Purity (4) under Ozagrel Sodium.

Bacterial endotoxins <*4.01*> Less than 3.7 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirements 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 Dissolve an amount of Ozagrel Sodium for Injection, equivalent to about 0.4 g of ozagrel sodium ($C_{13}H_{11}N_2NaO_2$), in water to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and 5 mL of water, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ozagrel Sodium Reference Standard, 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. Then, proceed as directed in the Assay under Ozagrel Sodium.

$$\text{Amount (mg) of ozagrel sodium } (C_{13}H_{11}N_2NaO_2) = W_S \times (Q_T/Q_S) \times 16$$

W_S: Amount (mg) of Ozagrel Sodium Reference Standard

Internal standard solution—A solution of benzoic acid in methanol (1 in 100).

Containers and storage Containers—Hermetic containers.

Papaverine Hydrochloride Injection

パパベリン塩酸塩注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 6.0 EU/mg.

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.

Add the following:

Peplomycin Sulfate for Injection

注射用ペプロマイシン硫酸塩

Peplomycin Sulfate for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Method of preparation Prepare as directed under Injections, with Peplomycin Sulfate.

Description Peplomycin Sulfate for Injection occurs as white light masses or powder.

Identification Take an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, and dissolve in 15 μL of Copper (II) sulfate TS and water to make 2 mL. Apply this solution to the column (prepared by filling a 15 mm inside diameter and 15 cm long chromatography tube with 15 mL of strongly basic ion exchange resin (Cl type) for column chromatography (75 – 150 μm in particle diameter) and run off. Then wash the column using water at 2.5 mL per minute, collect about 30 mL of the effluent. Add water to the effluent to make 250 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 242 nm and 246 nm, and between 291 nm and 295 nm. Further determine the absorbances A_1 and A_2, at 243 nm and 293 nm, respectively: the ratio A_1/A_2 is 1.20 to 1.30.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 50 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is 4.5 to 6.0.

Purity Clarity and color of solution—A solution prepared by dissolving an amount of Peplomycin Sulfate for Injection, equivalent to 10 mg (potency) of Peplomycin Sulfate according to the labeled amount, in 10 mL of water is clear and colorless.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Perform the sampling preventing from moisture absorption.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.5 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 organism, culture medium, liquid medium for suspending test organisms, preparation of seeded agar layer, preparation of cylinder-agar plate and the standard solutions —Proceed as directed in the Assay under Peplomycin Sulfate.

(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 containers of Peplomycin Sulfate for Injection. Weigh accurately an amount of the contents, equivalent to about 10 mg (potency) of Peplomycin Sulfate, dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8, to make exactly 100 mL. Measure exactly a suitable quantity of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 so that each mL contains 4 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.

Pethidine Hydrochloride Injection

ペチジン塩酸塩注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 6.0 EU/mg.

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

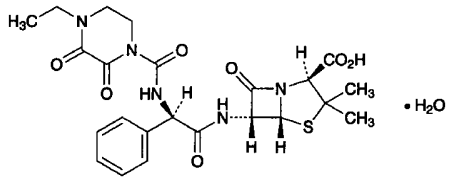
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Piperacillin Hydrate

ピペラシリン水和物



$C_{23}H_{27}N_5O_7S.H_2O$: 535.57

(2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid monohydrate [*66258-76-2*]

Piperacillin Hydrate contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Hydrate is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description Piperacillin Hydrate occurs as a white crystalline powder.

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

Identification **(1)** Determine the infrared absorption spectrum of Piperacillin Hydrate 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 Piperacillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the spectrum of a solution of Piperacillin Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 3) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ (^{1}H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triple signal A at about δ 1.1 ppm, a single signal B at about δ 4.2 ppm, and a multiple signal C at about δ 7.4 ppm, and the ratio of the integrated intensity of each signal, A:B:C, is about 3:1:5.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +162 – +172° (0.2 g, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metal ⟨*1.07*⟩—Proceed with 2.0 g of Piperacillin 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 1—Conduct this procedure rapidly after the preparation of the sample solution and standard solution. Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks, having the relative retention time of about 0.38 and about 0.50 with respect to piperacillin, obtained from the sample solution is not larger than twice the peak area of piperacillin from the standard solution (2), the total area of the peaks, having the relative retention time of about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (2), and the area of the peak other than piperacillin and other than the peaks having the relative retention time of about 0.38, about 0.50, about 0.82 and about 0.86 with respect to piperacillin, obtained from the sample solution, is not larger than the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than piperacillin obtained from the sample solution is not larger than the peak area of piperacillin from the standard solution (1).

Operating conditions—

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

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

System suitability—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin 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 (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 3.0%.

(3) Related substances 2—Dissolve 20 mg of Piperacillin Hydrate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 2 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, and the standard solutions (1) and (2) as directed under Liq-

uid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, obtained from the sample solution is not larger than three times the peak area of piperacillin from the standard solution (2), and the area of the peaks other than the peak of piperacillin and the peak having the relative retention time of about 6.6 with respect to piperacillin from the sample solution are not larger than 1.4 times the peak area of piperacillin from the standard solution (2). Furthermore, the total area of the peaks other than the peak of piperacillin from the sample solution is not larger than the area of the peak of piperacillin from the standard solution (1). For these calculations, use the area of the peak, having the relative retention time of about 6.6 with respect to piperacillin, after multiplying by the relative response factor, 2.0.

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 300 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 1.2 minutes.

Time span of measurement: About 8 times as long as the retention time of piperacillin, beginning after the piperacillin peak.

System suitability—

Test for required detectability: Confirm that the peak area of piperacillin obtained from 20 μL of the standard solution (2) is equivalent to 15 to 25% of that from 20 μL of the standard solution (1).

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 4.0%.

(4) Residual solvents <2.46>—Transfer exactly 10 mg of Piperacillin Hydrate to an about 3 mL-vial, add exactly 1 mL of saturated sodium hydrogen carbonate solution to dissolve and stop the vial tightly. After heating this at 90°C for 10 minutes, use the gas inside the container as the sample gas. Separately, measure exactly 1 mL of ethyl acetate, dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 20 mL. Pipet 2 μL of this solution in an about 3-mL vial containing exactly 1 mL of saturated sodium hydrogen carbonate solution, and stop the vial tightly. Run the procedure similarly to the sample, and use the gas as the standard gas. Perform the test with exactly 0.5 mL each of the sample gas and standard gas as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area of ethyl acetate by the automatic integration method: the peak area of ethyl acetate obtained from the sample gas is not larger than that from the standard gas.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous stylene-divinyl benzene copolymer for gas chromatography (average pore diameter of 0.0085 μm, 300 – 400 m^2/g) with the particle size of 125 to 150 μm.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 4 minutes.

System suitability—

System performance: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL each of ethyl acetate solution (1 in 400) and acetone solution (1 in 400), and stop the vial tightly. When the procedure is run under the above operating conditions, acetone and ethyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Take 1 mL of saturated sodium hydrogen carbonate solution in an about 3 mL-vial, add 2 μL of ethyl acetate solution (1 in 400), stop the vial tightly, and perform the test under the above operating conditions. When the procedure is repeated 6 times, the relative standard deviation of the peak area of ethyl acetate is not more than 10%.

Water <2.48> Not less than 3.2% and not more than 3.8% (0.5 g, volumetric titration, direct titration).

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

Bacterial endotoxins <4.01> Less than 0.07 EU/mg (potency).

Assay Weigh accurately an amount of Piperacillin Hydrate and Piperacillin Reference Standard, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. 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 height of piperacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of piperacillin } (C_{23}H_{27}N_5O_7S) = W_S \times (Q_T/Q_S) \times 1000$$

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

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Take 60.1 g of acetic acid (100) and 101.0 g of triethylamine, add water to make 1000 mL. To 25 mL of this solution add 210 mL of acetonitrile and 25 mL of dilute acetic acid, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin 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 internal standard and piperacillin 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 height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム

Add the following next to Loss on drying:

Bacterial endotoxins <*4.01*> Less than 2.4 EU/mg of prednisolone ($C_{21}H_{28}O_5$).

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.

Protamine Sulfate

プロタミン硫酸塩

Change the origin/limits of content to read:

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to the family *Salmonidae*.

It has a property to bind with heparin.

It binds with not less than 100 Units of heparin per mg, calculated on the dried basis.

Change the Description to read:

Description Protamine Sulfate occurs as a white powder. It is sparingly soluble in water.

Add the following next to Identification:

pH <*2.54*> Dissolve 1.0 g of Protamine Sulfate in 100 mL of water: the pH of this solution is between 6.5 and 7.5.

Change the Purity (2) to read

Purity (2) Absorbance—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance between 260 nm and 280 nm is not more than 0.1.

Delete the Potency as antiheparin, and add the following next to Purity:

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 3 hours).

Nitrogen content Weigh accurately about 10 mg of Protamine Sulfate, and perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N:14.01) is 22.5 – 25.5%, calculated on the dried basis.

Heparin-binding capacity

(i) Sample solution (a)—Weigh accurately about 15 mg of Protamine Sulfate, and dissolve in water to make exactly 100 mL. Repeat this procedure 3 times, and use the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

(ii) Sample solution (b)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 5 mL of water to them, and use these solutions as the sample solutions (b_1), (b_2) and (b_3).

(iii) Sample solution (c)—Pipet 10 mL each of the sample solutions (a_1), (a_2) and (a_3), add exactly 20 mL of water to them, and use these solutions as the sample solutions (c_1), (c_2) and (c_3).

(iv) Standard solution—Dissolve Heparin Sodium Reference Standard in water to make a solution containing exactly about 20 Units per mL.

(v) Procedure—Transfer exactly 2 mL of the sample solution to a cell for spectrophotometer, add the standard solution dropwise while mixing, and determine the transmittance at 500 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Continue the addition until a sharp change in the transmittance is observed, and note the volume, V mL, of the standard solution added. Repeat this procedure 2 times for each sample solution.

(vi) Calculation—Calculate the amount of heparin bound with 1 mg of the sample by the following formula from the volume of titrant on each sample solution, and calculate the average of 18 results obtained. The assay is not valid unless each relative standard deviation of 6 results ob-

tained from the sample solution (a), sample solution (b) and sample solution (c) is not more than 5%, respectively, and also unless each relative standard deviation of 6 results obtained from 3 sets, (a_1, b_1, c_1), (a_2, b_2, c_2) and (a_3, b_3, c_3) is not more than 5%, respectively.

Amount (heparin Unit) of heparin bound to 1 mg of Protamine Sulfate

$= S \times V \times (50/W_T) \times d$

S: Amount (heparin Unit) of heparin sodium in 1 mL of the standard solution

W_T: Amount (mg) of the sample, calculated on the dried basis

d: Dilution factor for each sample solution from the sample solution (a)

Sulfate content Weigh accurately about 0.15 g of Protamine Sulfate, dissolve in 75 mL of water, add 5 mL of 3 mol/L hydrochloric acid TS, and heat to boil. Add gradually 10 mL of barium chloride TS while boiling, and allow to stand for 1 hour while heating. Filter the precipitate formed, wash the precipitate with warm water several times, and transfer the precipitate into a tared crucible. Dry the precipitate, and incinerate by ignition to constant mass: the amount of sulfate (SO_4) is 16 – 22%, calculated on the dried basis, where 1 g of the residue is equivalent to 0.4117 g of SO_4.

Protamine Sulfate Injection

プロタミン硫酸塩注射液

Delete the Potency as antiheparin, and add the following next to Extractable volume:

Heparin-binding capacity Proceed the test as directed in the Heparin-binding capacity under Protamine Sulfate, changing the sample solution (a) as below: it binds with not less than 100 Units of heparin per mg of the labeled amount.

(i) Sample solution (a)—Pipet as amount of Protamine Sulfate Injection, equivalent to 15.0 mg of Protamine Sulfate according to the labeled amount, add water to make exactly 100 mL. Repeat this procedure more 2 times, and use the solutions so obtained as the sample solutions (a_1), (a_2) and (a_3).

Pyridoxine Hydrochloride Injection

ピリドキシン塩酸塩注射液

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 Membrane filtration method: it meets the requirement.

Reserpine Injection

レセルピン注射液

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 Membrane filtration method: it meets the requirement.

Riboflavin Sodium Phosphate Injection

リボフラビンリン酸エステルナトリウム注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/mg.

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.

Ringer's Solution

リンゲル液

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 Membrane filtration method: it meets the requirement.

Add the following:

Rokitamycin Tablets

ロキタマイシン錠

Rokitamycin Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$: 827.99).

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

Identification Take an amount of powdered Rokitamycin Tablets, equivalent to 10 mg (potency) of Rokitamycin according to the labeled amount, add 20 mL of methanol, and centrifuge if necessary. To 1 mL of this solution add methanol to make 25 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 230 nm and 233 nm.

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

Add 50 mL of water to 1 tablet of Rokitamycin Tablets, and disintegrate. Then add 10 mL of methanol, shake well, and add water to make exactly 100 mL. Centrifuge this solution if necessary, filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μg (potency) of Rokitamycin, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Rokitamycin Reference Standard, dissolve in 10 mL of methanol, and add 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, at 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/10)$

W_S: Amount [mg (potency)] of Rokitamycin 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 Rokitamycin Tablets is not less than 80%.

Start the test with 1 tablet of Rokitamycin 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 10 mL of the first 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 Rokitamycin according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Rokitamycin Reference Standard, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 232 nm of the sample solution and standard solution using water as the blank, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of rokitamycin ($C_{42}H_{69}NO_{15}$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$

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

C: Labeled amount [mg (potency)] of rokitamycin ($C_{42}H_{69}NO_{15}$) in 1 tablet

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ under the following conditions.

(i) Test organism, culture medium and standard solutions—Proceed as directed in the Assay under Rokitamycin.

(ii) Sample solutions—Weigh accurately not less than 20 tablets of Rokitamycin Tablets, and powder. Weigh accurately an amount of contents, equivalent to about 40 mg (potency) of Rokitamycin, add 50 mL of methanol, shake vigorously, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and centrifuge if necessary. Measure exactly a suitable quantity of this solution, add polysorbate 80 solution, prepared by adding 0.1 mol/L phosphate buffer solution, pH 8.0 to 0.1 g of polysorbate 80 to make 1000 mL, so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Roxithromycin

ロキシスロマイシン

Change the origin/limits of content to read:

Roxithromycin is a derivative of erythromycin.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Roxithromycin is expressed as mass (potency) of roxithromycin ($C_{41}H_{76}N_2O_{15}$).

Salicylic Acid

サリチル酸

Change the origin/limits of content to read:

Salicylic Acid, when dried, contains not less than 99.5% and not more than 101.0% of $C_7H_6O_3$.

Change the Description to read:

Description Salicylic Acid occurs as white crystals or crystalline powder. It has a slightly acid, followed by an acrid taste.

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

Change the Identification to read:

Identification **(1)** A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests <*1.09*> (1) and (3) for salicylate.

(2) Determine the absorption spectrum of a solution of Salicylic Acid in ethanol (95) (3 in 200,000) 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 Salicylic 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.

Change the Purity to read:

Purity **(1)** Chloride <*1.03*>—Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to 100 mL, and filter. Discard the first 20 mL of the filtrate, take subsequent 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.008%).

(2) Sulfate <*1.14*>—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and 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 VS (not more than 0.019%).

(3) Heavy metals <*1.07*>—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide TS, 2 mL of dilute acetic acid and water to make 50 mL, and 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 25 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Related substances—Dissolve 0.50 g of Salicylic Acid in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve exactly 10 mg of phenol, exactly 25 mg of 4-hydroxyisophthalic acid and exactly 50 mg of parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol obtained from the sample solution are not larger than the area of each respective peak from the standard solution, the area of the peak other than salicylic acid and other than the substances mentioned above is not larger than the peak area of 4-hydroxisophthalic acid from the standard solution, and the total area of peaks other than salicylic acid is not larger than 2 times the peak area of parahydroxybenzoic acid 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 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 water, methanol and acetic acid (100) (60:40:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of salicylic acid, 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 10 mL. Confirm that the peak areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol obtained from 10 μL of this solution are equivalent to 14 to 26% of the area of each respective peak from 10 μL of the standard solution.

System performance: Dissolve 10 mg of phenol, 25 mg of 4-hydroxyisophthalic acid and 50 mg of parahydroxybenzoic acid in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol are eluted in this order with the resolution between the peaks of 4-hydroxyisophthalic acid and phenol 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 areas of parahydroxybenzoic acid, 4-hydroxyisophthalic acid and phenol is not more than 2.0%, respectively.

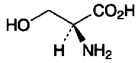
Change the Residue on ignition to read:

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Add the following:

L-Serine

L-セリン



$C_3H_7NO_3$: 105.09
(2*S*)-2-Amino-3-hydroxypropanoic acid [*56-45-1*]

L-Serine, when dried, contains not less than 98.5 and not more than 101.0% of $C_3H_7NO_3$.

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

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

It dissolves in 2 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Serine 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*⟩ $[\alpha]_D^{20}$: +14.0 – +16.0° (After drying, 2.5 g, 2 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.0 g of L-Serine in 10 mL of water is between 5.2 and 6.2.

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

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Serine. 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-Serine. 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-Serine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of L-Serine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Serine 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—Dissolve 0.10 g of L-Serine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under 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. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on Ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.11 g of L-Serine, 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
= 10.51 mg of $C_3H_7NO_3$

Containers and storage Containers—Tight containers.

Sodium Bicarbonate Injection

炭酸水素ナトリウム注射液

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 Membrane filtration method: it meets the requirement.

10% Sodium Chloride Injection

10% 塩化ナトリウム注射液

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 Membrane filtration method: it meets the requirement.

Sodium Citrate Injection for Transfusion

輸血用クエン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 Membrane filtration method: it meets the requirement.

Add the following:

Sodium Starch Glycolate

デンプングリコール酸ナトリウム

Sodium Starch Glycolate [*9063-38-1*]

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

Sodium Starch Glycolate is the sodium salt of a carboxymethyl ether of starch or of a cross-linked carboxymethyl ether of starch.

There are two neutralization types of Sodium Starch Glycolate, Type A and Type B, and their insoluble matter in a mixture of ethanol (99.5) and water (8:2), when dried, contains not less than 2.8% and not more than 4.2%, and not less than 2.0% and not more than 3.4% of sodium (Na: 22.99), respectively.

◆The label states the type of neutralization.◆

◆**Description** Sodium Starch Glycolate occurs as a white powder, and has a characteristic salty taste.

It practically insoluble in ethanol (99.5).

It swells with water, and becomes viscous, pasty liquid.

It is hygroscopic.◆

Identification **(1)** Acidify 5 mL of a solution of Sodium Starch Glycolate (1 in 500) with dilute hydrochloric acid, then add one drop of iodine TS, and stir: a blue to violet color is produced.

◆**(2)** Determine the infrared absorption spectrum of Sodium Starch Glycolate 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) The sample solution obtained in the Purity (2) responds to the Qualitative Tests <*1.09*> (2) for sodium salt. Perform the test using 2 mL of the sample solution and 4 mL of potassium hexahydroxoantimonate (V) TS.

pH <*2.54*> To 1 g of Sodium Starch Glycolate add 30 mL of water and stir: the pH of the resulting suspension of Type A is 5.5 – 7.5, and that of Type B is 3.0 – 5.0.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Sodium Starch Glycolate 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) Iron

(i) Sample solution Take 2.5 g of Sodium Starch Glycolate in a silica or platinum crucible, add 2 mL of 5 mol/L sulfuric acid TS. Heat on a water bath, then ignite cautiously with a gas burner or preferably in an electric furnace at 600 ± 25°C, and incinerate the residue completely. Allow to cool, add a few drops of 1 mol/L sulfuric acid TS, and heat and ignite as above. Allow to cool, add a few drops of ammonium carbonate TS, evaporate to dryness on a water bath, and heat and ignite as above. After cooling, dissolve the residue by adding 50 mL of water.

(ii) Standard solution Weigh accurately 863.4 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in water, add 25 mL of 1 mol/L sulfuric acid TS, and add water to make exactly 500 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 1.0 μg of iron (Fe).

(iii) Procedure Pipet 10 mL each of the sample solution and standard solution, and to each solution add 2 mL of citric acid solution (1 in 5) and 0.1 mL of thioglycolic acid. Then add ammonia solution (28) dropwise to render the solution alkaline, using litmus paper as an indicator. Add water to make 20 mL, and use these solutions as the test solution and the control solution, respectively. Allow these solutions to stand for 5 minutes, and compare the color of the solutions using white background: the color of the test solution is not deeper than that of the control solution (not more than 20 ppm).

(3) Sodium glycolate—Conduct this procedure without exposure to light, using light-resistant vessels.

(i) Sample solution Weigh accurately 0.200 g of Sodium Starch Glycolate in a beaker, add 4 mL of 6 mol/L acetic acid TS and 5 mL of water, and stir to dissolve. Add 50 mL of acetone and 1 g of sodium chloride, stir, and filter through a filter paper previously soaked with acetone. Rinse the beaker and the filter paper with acetone, combine the filtrate and washings, and add acetone to make exactly 100 mL. Allow to stand for 24 hours, and use the supernatant liquid as the sample solution.

(ii) Standard solution To exactly 0.310 g of glycolic acid, previously dried in a desiccator (silica gel) for 18 hours, add water to dissolve to make exactly 500 mL. Pipet 5 mL of this solution, add 4 mL of 6 mol/L acetic acid TS, and allow to stand for 30 minutes. Add 50 mL of acetone and 1 g of sodium chloride, proceed as (i) above, and use the supernatant liquid as the standard solution.

(iii) Procedure Pipet 2.0 mL each of the sample solution and standard solution into 25-mL stoppered test tubes,

and heat on a water bath for 20 minutes to remove acetone. After cooling, add 20.0 mL of 2,7-dihydroxynaphthalene TS to the residue, stopper the test tube, and heat on a water bath for 20 minutes. Cool under running water, and transfer whole quantity of the content to a 25-mL volumetric flask. Maintain the flask under running water, and add sulfuric acid to make 25 mL. Within 10 minutes, determine the absorbance of these solutions at 540 nm using water as the blank as directed under Ultraviolet-visible Spectrophotometry *<2.24>*; the absorbance of the sample solution is not larger than that of the standard solution (not more than 2.0%).

(4) Sodium chloride—Weigh accurately about 0.5 g of Sodium Starch Glycolate in a beaker, disperse in 100 mL of water, and add 1 ml of nitric acid. Titrate *<2.50>* with 0.1 mol/L silver nitrate VS (potentiometric titration): the amount of sodium chloride (NaCl: 58.44) is not more than 7.0%.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

Loss on drying *<2.41>* Not more than 10.0% (1 g, 130°C, 90 minutes).

Assay To about 1 g of Sodium Starch Glycolate add 20 mL of a mixture of ethanol (99.5) and water (8:2), stir for 10 minutes, and filter. Repeat this procedure until no more turbidity is produced by adding silver nitrate TS, and dry the residue on the filter paper at 105°C to constant mass. Weigh accurately 0.7 g of the mass, add 80 mL of acetic acid (100), and heat the mixture under a reflux condenser on a water bath for 2 hours. After cooling, titrate *<2.50>* with 0.1 mol/L perchloric acid VS (potentiometric titration).

$$\text{Content (\%) of sodium (Na)} = (V \times 2.299 \times 100)/W$$

V: Consumed amount (mL) of 0.1 mol/L perchloric acid VS

W: Mass (mg) of the dried residue

◆**Containers and storage** Containers—Tight containers.◆

Sodium Thiosulfate Injection

チオ硫酸ナトリウム注射液

Delete the Pyrogen and add the following next to Identification:

Bacterial endotoxins *<4.01>* Less than 0.01 EU/mg.

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.

Sulbactam Sodium

スルバクタムナトリウム

Change the origin/limits of content to read:

Sulbactam Sodium contains not less than 875 μg (potency) and not more than 941 μg (potency) per mg, calculated on the anhydrous basis. The potency of Sulbactam Sodium is expressed as mass (potency) of sulbactam ($C_8H_{11}NO_5S$: 233.24).

Delete the following two Monographs:

Sulfinpyrazone

スルフィンピラゾン

Sulfinpyrazone Tablets

スルフィンピラゾン錠

Sulpyrine Injection

スルピリン注射液

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 Membrane filtration method: it meets the requirement.

Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物

Change the origin/limits of content to read:

Sultamicillin Tosilate Hydrate contains not less than 698 μg (potency) and not more than 800 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of residual solvent. The potency of Sultamicillin Tosilate Hydrate is expressed as mass (potency) of sultamicillin ($C_{25}H_{30}N_4O_9S_2$: 594.66).

Suxamethonium Chloride for Injection

注射用スキサメトニウム塩化物

Add the following next to Purity:

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.5 EU/mg.

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.

Suxamethonium Chloride Injection

スキサメトニウム塩化物注射液

Add the following next to Purity:

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.0 EU/mg.

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.

Talc

タルク

Change to read:

Talc is a fractured and screened native hydrous magnesium silicate. Pure talc is $Mg_3Si_4O_{10}(OH)_2$: 379.27. Talc may contain related mineral substances consisting chiefly of chlorite (hydrous magnesium aluminum silicate), magnesite (magnesium carbonate), calcite (calcium carbonate) and dolomite (calcium magnesium carbonate).

It contains no asbestos.

It contains not less than 17.0% and not more than 19.5% of magnesium (Mg: 24.31).

Description Talc occurs as a white to grayish white, fine, crystalline powder.

It is unctuous, and adheres readily to the skin.

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

Identification Determine the infrared absorption spectrum of Talc as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3680 cm^{-1}, 1018 cm^{-1} and 669 cm^{-1}.

Purity **(1)** Acidity or alkalinity—To 2.5 g of Talc add 50 mL of freshly boiled and cooled water, and heat under a reflux condenser. Filter the liquid by suction, add 0.1 mL of bromothymol blue-sodium hydroxide-ethanol TS to 10 mL of the filtrate, and add 0.01 mol/L hydrochloric acid VS until the color of the solution changes: the necessary volume of the VS is not more than 0.4 mL. Separately, to 10 mL of the filtrate add 0.1 mL of phenolphthalein TS, and add 0.01 mol/L sodium hydroxide VS until the color of the solution changes to light red: the necessary volume of the VS is not more than 0.3 mL.

(2) Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of the filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800±25°C: the amount of the residue is not more than 2.0%.

(3) Water-soluble substances—To 10.0 g of Talc add 50 mL of water, weigh the mass, and boil for 30 minutes, supplying water lost by evaporation. Cool, add water to restore the original mass, and filter. Centrifuge, if necessary, until the filtrate becomes clear. Evaporate 20 mL of the filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 4.0 mg.

(4) Iron—Weigh accurately about 10 g of Talc, add 50 mL of 0.5 mol/L hydrochloric acid VS gently while stirring, and heat under a reflux condenser on a water bath for 30 minutes. After cooling, transfer the content to a flask, and allow to precipitate the insoluble matter. Filter the supernatant liquid through a filter paper for quantitative analysis (No. 5B), leaving the precipitate in the flask as much as possible, wash the remaining precipitate in the flask with three 10-mL portions of hot water, and also wash the filter paper with 15 mL of hot water, and combine the washings and the filtrate. After cooling, add water to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 2.5 mL of the stock solution, add 50 mL of 0.5 mol/L hydrochloric acid VS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 50 mL each of 0.5 mol/L hydrochloric acid VS add exactly 2 mL, 2.5 mL, 3 mL and 4 mL of Standard Iron Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions, and calculate the amount of iron from the calibration curve prepared from the absorbances of the stan-

dard solutions: not more than 0.25%.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Iron hollow-cathode lamp.
Wavelength: 248.3 nm.

(5) Aluminum—Pipet 5 mL of the sample stock solution obtained in the Assay, add 10 mL of cesium chloride TS and 10 mL of hydrochloric acid, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of cesium chloride TS add exactly 5 mL, 10 mL, 15 mL and 20 mL of Standard Aluminum Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of aluminum from the calibration curve prepared from the absorbances of the standard solutions: not more than 2.0%.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.
Lamp: Aluminum hollow-cathode lamp.
Wavelength: 309.3 nm.

(6) Lead—Use the sample stock solution obtained in (4) as the sample solution. Separately, to 50 mL of 0.5 mol/L hydrochloric acid VS add exactly 5 mL, 7.5 mL, 10 mL and 12 mL of Standard Lead Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of lead from the calibration curve prepared from the absorbances of the standard solutions: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 217.0 nm.

(7) Calcium—Pipet 2.5 mL of the sample stock solution obtained in the Assay, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 1 mL, 2 mL, 3 mL and 4 mL of Standard Calcium Solution, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of calcium from the calibration curve prepared from the absorbances of the standard solutions: not more than 0.9%.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

(8) Arsenic <*1.11*>—To 0.5 g of Talc add 5 mL of dilute sulfuric acid, and heat gently to boiling with shaking. Cool immediately, filter, and wash the residue with 5 mL of dilute sulfuric acid, then with 10 mL of water. Combine the filtrate and the washings, evaporate to 5 mL on a water bath, and perform the test with this solution as the test solution (not more than 4 ppm).

Loss on ignition <*2.43*> Not more than 7.0% (1 g, 1050 – 1100°C, constant mass).

Assay Weigh accurately about 0.5 g of Talc in a polytetrafluoroethylene dish, add 5 mL of hydrochloric acid, 5 mL of nitric acid and 5 mL of perchloric acid, then add 35 mL of hydrofluoric acid while mixing gently, and evaporate to dryness on a hot plate by heating gradually. Add 5 mL of hydrochloric acid to the residue, cover the dish with a watch glass, and heat to boil. After cooling, transfer the content to a volumetric flask while washing the watch glass and dish with water, further wash the dish with water, transfer the washings to the flask, then add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 0.5 mL of the sample stock solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, to 10 mL of hydrochloric acid and 10 mL of lanthanum chloride TS add exactly 2.5 mL, 3 mL, 4 mL and 5 mL of Standard Magnesium Solution for Atomic Absorption Spectrophotometry, add water to make them exactly 100 mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of magnesium from the calibration curve prepared from the absorbances of the standard solutions.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Magnesium hollow-cathode lamp.
Wavelength: 285.2 nm.

Containers and storage Containers—Well-closed containers.

Teceleukin for Injection (Genetical Recombination)

注射用テセロイキン(遺伝子組換え)

Change the Loss on drying to read:

Loss on drying Transfer the content of the vial of Teceleukin for Injection (Genetical Recombination) to a weighing bottle under the atmosphere not exceeding 10% relative humidity, and perform the test as directed in the Water content determination described in the Minimum Requirements for Biological Products: not more than 5%.

Thiamine Chloride Hydrochloride Injection

チアミン塩化物塩酸塩注射液

Add the following next to Identification:

Bacterial endotoxins ⟨*4.01*⟩ Less than 6.0 EU/mg.

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.

Thiopental Sodium for Injection

注射用チオペンタールナトリウム

Add the following next to Loss on drying:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.30 EU/mg.

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.

Tipepidine Hibenzate Tablets

チペピジンヒベンズ酸塩錠

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 Tipepidine Hibenzate Tablets add 5 mL of diluted acetic acid (100) (1 in 2) and 15 mL of methanol per 11 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and warm for 15 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly V mL so that each mL contains about 0.44 mg of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$), and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tipepidine hibenzate ($C_{15}H_{17}NS_2.C_{14}H_{10}O_4$) $= W_S \times (Q_T/Q_S) \times (V/50)$

W_S: Amount (mg) of tipepidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Add the following:

Tobramycin Injection

トブラマイシン注射液

Tobramycin Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of tobramycin ($C_{18}H_{37}N_5O_9$: 467.51).

Method of preparation Prepare as directed under Injections, with Tobramycin.

Description Tobramycin Injection occurs as a colorless or very pale yellow clear liquid.

Identification To a volume of Tobramycin Injection, equivalent to 10 mg (potency) of Tobramycin according to the labeled amount, add water to make 1 mL, and use this solution as the sample solution. Separately, dissolve 10 mg (potency) of Tobramycin Reference Standard in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Tobramycin.

Osmotic pressure ratio Being specified separately.

pH ⟨*2.54*⟩ 5.0 – 7.0

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 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 Tobramycin.

(ii) Sample solutions—To exactly 5 mL of Tobramycin Injection add 0.1 mol/L phosphate buffer solution, pH 8.0 so that each mL contains 1 mg (potency) of Tobramycin. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions

so that each mL contains 8 μg (potency) and 2 μg (potency), and use these solutions as the high and low concentration sample solutions, respectively.

Containers and storage Containers—Hermetic containers.

Trimetazidine Hydrochloride

トリメタジジン塩酸塩

Change the Purity (2) to read:

Purity (2) Related substances—Dissolve 0.2 g of Trimetazidine Hydrochloride in 50 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample 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 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 each peak area by the automatic integration method: the area of the peak other than trimetazidine obtained from the sample solution is not larger than 1.5 times that of trimetazidine from the standard solution, and the total area of the peaks other than trimetazidine from the sample solution is not larger than 2.5 times the peak area of trimetazidine 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 40°C.

Mobile phase A: Dissolve 2.87 g of sodium 1-heptanesulfonate in water to make 1000 mL, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10). Mix 3 volumes of this solution and 2 volumes of methanol.

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 – 50	95→75	5 →25

Flow rate: Adjust the flow rate so that the retention time of trimetazidine is about 25 minutes.

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

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of trimetazidine obtained from 10 μL of this solution is equivalent to 18 to 32% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 15,000 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 trimetazidine is not more than 2.0%.

Delete the following two Monographs:

Tubocurarine Chloride Hydrochloride Hydrate

ツボクラリン塩化物塩酸塩水和物

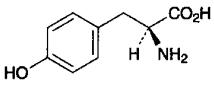
Tubocurarine Chloride Hydrochloride Injection

ツボクラリン塩化物塩酸塩注射液

Add the following:

L-Tyrosine

L-チロジン



$C_9H_{11}NO_3$: 181.19

(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoic acid

[*60-18-4*]

L-Tyrosine, when dried, contains not less than 99.0 % and not more than 101.0% of $C_9H_{11}NO_3$.

Description L-Tyrosine occurs as white crystals or a crystalline powder.

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

It dissolves in dilute hydrochloric acid and in ammonia TS.

Identification (1) Determine the absorption spectrum of a solution of L-Tyrosine in 0.1 mol/L hydrochloric acid (1 in 10,000) 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 L-Tyrosine 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*⟩ $[\alpha]_D^{20}$: −10.5 – −12.5° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Tyrosine in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of L-Tyrosine in 12 mL of dilute nitric acid and 20 mL of water, and add 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 12 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Tyrosine in 5 mL of dilute hydrochloric acid, and add water to make 45 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 5 mL of dilute hydrochloric acid and water to make 45 mL. To the test solution and the control solution add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Tyrosine. 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-Tyrosine 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-Tyrosine according to Method 3, 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—Dissolve 0.20 g of L-Tyrosine in 10 mL of diluted ammonia solution (28) (1 in 2), add water to make 20 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under 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. Then develop with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.18 g of L-Tyrosine previously dried, dissolve in 6 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.

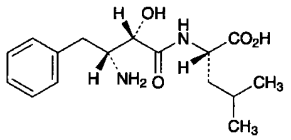
Each mL of 0.1 mol/L perchloric acid VS
= 18.12 mg of $C_9H_{11}NO_3$

Containers and storage Containers—Tight containers.

Add the following:

Ubenimex

ウベニメクス



$C_{16}H_{24}N_2O_4$: 308.37
(2*S*)-2-[(2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoylamino]-4-methylpentanoic acid
[*58970-76-6*]

Ubenimex, when dried, contains not less than 98.5 % and not more than 101.0% of $C_{16}H_{24}N_2O_4$.

Description Ubenimex occurs as a white crystalline powder.

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

It dissolves in 1 mol/L hydrochloric acid TS.

Melting point: about 230°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ubenimex (1 in 2000) 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 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*⟩ $[\alpha]_D^{20}$: −15.5 – −17.5° (after drying, 0.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Ubenimex 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 Ubenimex in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase A to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the

area of the peak other than ubenimex obtained from the sample solution is not larger than 1/2 times the peak area of ubenimex from the standard solution. Furthermore, the total area of the peaks other than ubenimex from the sample solution is not larger than the peak area of ubenimex 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 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase A: A mixture of diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) and acetonitrile for liquid chromatography (17:3).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and diluted 0.1 mol/L potassium dihydrogen phosphate TS (13 in 20) (2:1).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	100	0
20 – 60	100→0	0→100
60 – 70	0	100

Flow rate: Adjust the flow rate so that the retention time of ubenimex is about 14 minutes.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of ubenimex obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ubenimex are not less than 5000 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 ubenimex is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ubenimex, previously dried, dissolve in 60 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
= 30.84 mg of $C_{16}H_{24}N_2O_4$

Containers and storage Containers—Tight containers.

Vincristine Sulfate

ビンクリスチン硫酸塩

Change the Description to read:

Description Vincristine Sulfate occurs as a white to light yellowish white powder.

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

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: +28.5 – +35.5° (0.2 g, calculated on the dried basis, water, 10 mL, 100 mm).

Change the Identification to read:

Identification

(1) Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vincristine Sulfate 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 Vincristine Sulfate 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 Vincristine Sulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

Change the Purity to read:

Purity **(1)** Clarity and color of solution—Dissolve 50 mg of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 10 mg of Vincristine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 200 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 peak area of desacetyl vincristine and vinblastine, having the relative retention times of about 0.9 and about 1.6 with respect to vincristine, respectively, obtained

from the sample solution are not larger than 1/8 times and 3/20 times, respectively, the peak area of vincristine from the standard solution, and the area of the peak other than vincristine, desacetyl vincristine and vinblastine from the sample solution is not larger than 1/4 times the peak area of vincristine from standard solution. Furthermore, the total area of the peaks other than vincristine from the sample solution is not larger than the peak area of vincristine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

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

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

Mobile phase A: methanol.

Mobile phase B: A mixture of water and diethylamine (197:3), adjusted the pH to 7.5 with phosphoric acid.

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 – 12	62	38
12 – 27	62→92	38→8

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 15 minutes.

Time span of measurement: About 1.7 times as long as the retention time of vincristine, 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 200 mL. Confirm that the peak area of vincristine obtained from 200 μL of this solution is equivalent to 1.75 to 3.25% of that from 200 μL of the standard solution.

System performance: Dissolve 15 mg each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. When the procedure is run with 200 μL of this solution under the above operating conditions, vincristine and vinblastine 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 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vincristine is not more than 1.5%.

Change the Loss on drying to read:

Loss on drying Perform the test with about 10 mg of Vincristine Sulfate as directed in Method 2 under Thermal Analysis ⟨*2.52*⟩ according to the following conditions: not more than 12.0%.

Operating conditions—

Heating rate: 5°C/minute.

Temperature range: room temperature to 200°C.

Atmospheric gas: dried nitrogen.

Flow rate of atmospheric gas: 40 mL/minute.

Change the Assay to read:

Assay Weigh accurately about 10 mg each of Vincristine Sulfate and Vincristine Sulfate Reference Standard (separately determine the loss on drying in the same manner as Vincristine Sulfate), dissolve each in water to make exactly 10 mL, and use these solutions as the sample solution and the 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 vincristine peak areas, A_T and A_S, of both solutions.

Amount (mg) of $C_{46}H_{56}N_4O_{10}.H_2SO_4 = W_S \times (A_T/A_S)$

W_S: Amount (mg) of Vincristine Sulfate Reference Standard, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

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

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

Mobile phase: Adjust the pH to 7.5 of a mixture of water and diethylamine (59:1) with phosphoric acid. To 300 mL of this solution add 700 mL of methanol.

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

System suitability—

System performance: Dissolve 5 mg each of Vincristine Sulfate and vinblastine sulfate in 5 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, vincristine and vinblastine 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 vincristine is not more than 1.0%.

Change the Containers and storage to read:

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at not exceeding −20°C.

Water for Injection

注射用水

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.

Xylitol Injection

キシリトール注射液

Add the following next to Extractable volume:

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

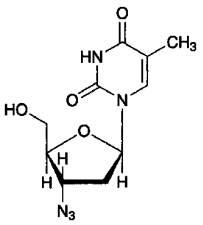
Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Add the following:

Zidovudine

ジドブジン



$C_{10}H_{13}N_5O_4$: 267.24

3′-Azido-3′-deoxythymidine [*30516-87-1*]

Zidovudine contains not less than 97.0% and not more than 102.0% of $C_{10}H_{13}N_5O_4$, calculated on the anhydrous basis.

Description Zidovudine occurs as a white to pale yellowish white powder.

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

It gradually turns yellow-brown on exposure to light.

Melting point: about 124°C.

Identification Determine the infrared absorption spectrum of Zidovudine as directed in the potassium bromide disc method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Zidovudine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the Reference Standard separately in a small amount of water and dry them in a desiccator (in vacuum, phosphorus (V) oxide), and perform the test with the residues.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: +60.5 – +63.0° (0.5 g calculated on the anhydrous basis, ethanol (99.5), 50 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩— Proceed with 1.0 g of Zidovudine 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) 1-[(2*R*,5*S*)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl]thymine, triphenylmethanol, and other related substances—Dissolve 0.20 g of Zidovudine in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, add 1 mL of the sample solution to 20 mg each of thymine for liquid chromatography, 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine for thin-layer chromatography, and triphenylmethanol for thin-layer chromatography, and add methanol to dissolve to make exactly 100 mL. Pipet 5 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 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 chloroform and methanol (9:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution that corresponds to the position of the 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine obtained from the standard solution is not more intense than the spot from the standard solution, and the spot other than the principal spot and spots other than thymine and 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine from the sample solution is not more intense than zidovudine spot from the standard solution. However, the 3 spots from the standard solution appear in ascending order of *R*f value thymine, 1-[(2*R*,5*S*)-2,5-dihydro-5-(hydroxymethyl)-2-furyl]thymine, and zidovudine. Furthermore, spray evenly on the plate a solution of vanillin in sulfuric acid (1 in 100): the spot from the sample solution corresponding to the spot of triphenylmethanol from the standard solution is not more intense than the spot from the standard solution.

(3) Thymine, 3′-chloro-3′-deoxythymidine, and other related substances—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 20 mg of thymine for liquid chromatography, dissolve in 100 mL of methanol, and add the mobile phase to make exactly 250 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the thymine peak areas, A_T and A_S, of the sample and standard solutions, and calculate the amount of thymine using the following formula: the amount is not more than 2.0%. Also, determine the peak area of each peak obtained from the sample solution by the automatic integration method, and calculate the amounts of related substances other than thymine by the area percentage method: the amount of 3′-chloro-3′-deoxythymidine, whose

relative retention time to zidovudine is 1.2, is not more than 1.0%, and is not more than 0.5% for all other related substances. Finally, the total amount of thymine, 3′-chloro-3′-deoxythymidine, and all related substances obtained above is not more than 3.0%.

$$\text{Amount (\%) of thymine} = (W_S/W_T) \times (A_T/A_S) \times 10$$

W_S: Amount (mg) of thymine for liquid chromatography
W_T: Amount (mg) of Zidovudine

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 zidovudine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of zidovudine obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that from 10 μL of the solution for system suitability test.

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

Water ⟨*2.48*⟩ Not more than 1.0% (0.25 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g).

Assay Weigh accurately about 50 mg of Zidovudine and Zidovudine Reference Standard (separately determine the water ⟨*2.48*⟩ using the same manner as Zidovudine), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of each solution, add the mobile phase to make them exactly 50 mL, and use these solutions as the sample and standard solutions, 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. Determine the peak areas of zidovudine, A_T and A_S of both solutions.

$$\text{Amount (mg) of } C_{10}H_{13}N_5O_4 = W_S \times (A_T/A_S)$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

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

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

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of zidovudine is about 15 minutes.

System suitability—

System performance: Dissolve 50 mg of Zidovudine in 50 mL of the mobile phase. Separately, dissolve 5 mg of 3′-chloro-3′-deoxythymidine for liquid chromatography in 50 mL of the mobile phase. Mix 10 mL and 1 mL of these solutions, respectively, and add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above conditions, zidovudine and 3′-chloro-3′-deoxythymidine are eluted in this order with the resolution between these peaks being not less than 1.4, and the symmetry factor of the peak of zidovudine is not more than 1.5.

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

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Crude Drugs

Alpinia Officinarum Rhizome

リョウキョウ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Alpinia Officinarum 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 Alpinia Officinarum Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Anemarrhena Rhizome

チモ

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Anemarrhena 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 Anemarrhena Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of fiber, originating from the dead leaves, and other foreign matters contained in Anemarrhena Rhizome is not more than 3.0%.

Angelica Dahurica Root

ビャクシ

Change the Purity to read:

Purity **(1)** Leaf sheath—The amount of leaf sheath contained in Angelica Dahurica Root does not exceed 3.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Angelica Dahurica 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).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Angelica Dahurica Root 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 leaf sheath contained in Angelica Dahurica Root is not more than 1.0%.

Apricot Kernel

キョウニン

Change the Identification to read:

Identification To 1.0 g of ground Apricot Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin 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 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. Examine under ultraviolet light (main wavelength: 365 nm): a spot with a blueish white fluorescence appears at around *R*f 0.7. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon 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-brown spot from the standard solution.

Add the following:

Aralia Rhizome

Araliae Cordatae Rhizoma

ドクカツ

Aralia Rhizome is usually the rhizome of *Aralia cordata* Thunberg (*Araliaceae*).

Description Aralia Rhizome is curved, irregular cylindrical to masses occasionally with remains of short roots. 4 to 12 cm in length, 2.5 to 7 cm in diameter, often cut crosswise or lengthwise. 1 to several, enlarged dents by remains of stems on the upper part or rarely 1.5 to 2.5 cm in diameter, remains of short stem. The outer surface is dark brown to yellowish brown, with longitudinally wrinkles, bases or dents of root. The transverse section of rhizome reveals dark brown to yellowish brown, scattered brownish small spots with oil canals, and with numerous splits.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of rhizome reveals the outermost layer to be cork layer, rarely composed of cork stone cells, followed these appeared sever-

al layers of collenchyma. Vascular bundle and medullary rays is distinct, pith broad. Phloem fibre bundles are sometimes observed at the outer portion of phloem. Oil canals composed of schizogenous intercellular space in cortex and pith. Cortex composed of vessels, xylem fibres, and occasionally thick-wall xylem parenchyma. Vascular bundles scattered on the pith. And, parenchymatous cells observed rosette aggregates of calcium oxalate. Starch grains composed of simple grains, 2- to 6- compound grains.

Identification To 1 g of pulverized Aralia Rhizome add 10 mL of methanol, shake for 5 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, ethyl acetate and acetic acid (100) (30:10:1) 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 for 5 minutes: a purple spot appears at around *R*f 0.6.

Loss on drying <*5.01*> Not more than 12.0%.

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 15.0%.

Asiasarum Root

サイシン

Change the Purity to read:

Purity **(1)** Terrestrial part—Any terrestrial parts are not found.

(2) Arsenic <*1.11*>—Prepare the test solution with 0.4 g of pulverized Asiasarum Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of foreign matter other than terrestrial part contained in Asiasarum Root is not more than 1.0%.

(4) Aristolochic acid I—To exactly 2.0 g of pulverized Asiasarum Root add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve exactly 1.0 mg of aristolochic acid I for crude drugs purity test in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, according to the following conditions: the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample solution shows such a peak, repeat the test under different conditions to confirm that the peak in question is not aristolochic acid I.

Operating conditions—

Detector: An ultraviolet or visible absorption photometer (wavelength: 400 nm).

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

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

Mobile phase: A mixture of a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 2 mL of phosphoric acid in water to make 1000 mL and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of aristolochic acid I is about 15 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add diluted methanol (3 in 4) to make exactly 10 mL. Confirm that the ratio, S/N, of the signal (S) and noise (N) of aristolochic acid I obtained from 20 μL of this solution is not less than 3. In this case, S means the peak height on the chromatogram not including noise obtained by drawing an average line of the detector output, and N is 1/2 of the difference between the maximum and minimum output signals of the baseline around the peak in the range of 20 times the width at half-height of the peak.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aristolochic acid I is not more than 5.0%.

(5) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Asparagus Tuber

テンモンドウ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Asparagus Tuber 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 Asparagus Tuber according to Method 4, and perform the test (not more than 5 ppm).

Atractylodes Rhizome

ビャクジュツ

Change the Purity to read:

Purity **(1)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(2) Atractylodes lancea rhizome—To 2.0 g of pulverized Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the solution on a plate of silica gel 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. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears at the *R*f value of between 0.3 and 0.6.

Powdered Atractylodes Rhizome

ビャクジュツ末

Change the Purity to read:

Purity **(1)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(2) Atractylodes lancea rhizome—To 2.0 g of Powdered Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the solution on a plate of silica gel 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. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears at the *R*f value of between 0.3 and 0.6.

Belladonna Extract

ベラドンナエキス

Add the following next to Identification:

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Belladonna Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Calumba

コロンボ

Add the following next to Identification:

Purity 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

Add the following next to Identification:

Purity 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).

Cimicifuga Rhizome

ショウマ

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cimicifuga 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 Cimicifuga Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Rhizome of *Astilbe thunbergii* Miquel—Under a microscope <*5.01*>, pulverized Cimicifuga Rhizome does not contain crystal druses in the parenchyma.

Clematis Root

イレイセン

Add the following next to Identification:

Purity Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Clematis Root according to Method 4, and perform the test (not more than 5 ppm).

Cnidium Rhizome

センキュウ

Add the following next to Description:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cnidium 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 Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Cnidium Rhizome

センキュウ末

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Cnidium 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 Powdered Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Cnidium Rhizome does not contain a large quantity of starch grains, stone cells, crystals of calcium oxalate or other foreign matter.

Condurango Fluidextract

コンズランゴ流エキス

Add the following next to Identification:

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Condurango Fluidextract as direct in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Coptis Rhizome

オウレン

Add the following next to Identification:

Purity Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Coptis Rhizome

オウレン末

Change the Purity to read:

Purity **(1)** Phellodendron bark—Under a microscope <*5.01*>, crystal cell rows or mucilage masses are not observable. Stir 0.5 g of Powdered Coptis Rhizome with 2 mL of water: the solution does not become gelatinous.

(2) Curcuma—Place Powdered Coptis Rhizome on a filter paper, drop diethyl ether on it, and allow to stand. Remove the powder from the filter paper, and drop 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <*5.01*>, Powdered Coptis Rhizome does not contain gelatinized starch or secretory cells containing yellow-red resin.

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Corydalis Tuber

エンゴサク

Change the Identification to read:

Identification To 2 g of pulverized Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a yellow-green fluorescent spot at around *R*f 0.4 and a yellow fluorescent spot at around *R*f 0.35 appear. When spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at around *R*f 0.6.

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Corydalis Tuber 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 Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

Add the following:

Powdered Corydalis Tuber

Corydalis Tuber Pulveratum

エンゴサク末

Powdered Corydalis Tuber is the powder of Corydalis Tuber.

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

Description Powdered Corydalis Tuber occurs as a greenish yellow to grayish yellow powder. Almost odorless; taste, bitter.

Under a microscope <*5.01*>, Powdered Corydalis Tuber reveals mainly, masses of gelatinized starch or light yellow to colorless parenchymatous cells containing starch grains, fragments of cork layers, light yellow stone cells, sclerenchymatous cells, reticulate vessels, spiral vessels and ring vessels; starch grains observed simple grains and 2- to 3-

compound grains.

Identification To 2 g of Powdered Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under 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 methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a yellow-green fluorescent spot and a yellow fluorescent spot appear at around *R*f 0.4 and at around *R*f 0.35, respectively. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at around *R*f 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Corydalis Tuber 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 0.4 g of Powdered Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 15.0%.

Total ash <*5.01*> Not more than 3.0%.

Component determination To about 1 g of Powdered Corydalis Tuber, accurately weighed, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of the mixture of methanol and dilute hydrochloric acid (3:1), and proceed in the same way as above. Combine the filtrate, add the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for component determination, previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 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 dehydrocorydaline.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate ($C_{22}H_{24}N_2O_7$)]
$= W_S \times (A_T/A_S) \times (1/4)$

W_S: Amount (mg) of dehydrocorydaline nitrate for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

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

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

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. Dissolve 14.05 g of sodium perchlorate monohydrate in this solution, and add water to make exactly 1000 mL. Add 450 mL of acetonitrile, and dissolve 0.20 g of sodium lauryl sulfate in this solution.

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

System suitability—

System performance: Dissolve 1 mg of dehydrocorydaline nitrate for component determination and 1 mg of berberine chloride in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dehydrocorydaline is not more than 1.5%.

Add the following:

Crataegus Fruit

Crataegi Fructus

サンザシ

Crataegus Fruit is the pseudocarp of **1)** *Crataegus cuneata* Siebold et Zuccarini or **2)** *Crataegus pinnatifida* Bunge var. *major* N. E. Brown (*Rosaceae*) without any treatment or cut crosswise or lengthwise.

Description

1) *Crataegus cuneata* Siebold et Zuccarini Nearly sphaerical fruits, 8 to 14 mm in diameter; externally yellowish brown to grayish brown, with fine reticulated wrinkles, remained dent of 4 to 6 mm in diameter at one end, often the base of calyx around the dent, short peduncle or scar at the other end. True fruits, usually five loculus, often split five, mericarp, 5 to 8 mm in length, light brown, usually, containing one seed into each mericarp.

Almost odorless; taste, slightly acid.

Under a microscope <*5.01*>, a transverse section of central parts reveals in the outermost layer composed of epidermis to be covered with comparatively thick cuticle layer, cuticle intrude into lateral cell walls of epidermis, and reveal wedge-like. Cell of the epidermis or 2- to 3-layer of parenchymatous cells beneath these observed contents of yellowish brown to red brown in color followed these appeared parenchyma. Vascular bundles and numerous stone cells appear single or gathered 2 to several cells scattered on the parenchyma, and observed solitary crystals and rosette aggregates

of calcium oxalate. Pericarp of true fruits composed of mainly sclerenchymatous cells, seed covered with seed coats, perisperm, endosperm, cotyledon observed inside seed coats; sclerenchymatous cells of true fruits and cells of seed coats containing solitary crystals of calcium oxalate.

2) *Crataegus pinnatifida* Bunge var. *major* N. E. Brown

Approximate to Crataegus Fruits 1), but it is a large size, 17 to 23 mm in diameter, the outer surface red brown and lustrous, spot-like scars of hairs are distinct. At one end remained dent, 7 to 9 mm in diameter, mericarp, 10 to 12 mm in length, yellowish brown in color, usually ripe seeds are absent.

Odor, characteristic; taste, acid.

Under a microscope <*5.01*>, a transverse section of the central parts approximate to 1), but it is a few stone cells on parenchyma.

Identification To 1 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hyperoside for thin-layer chromatography in 20 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, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 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 green fluorescent spot from the standard solution. This spot disappears gradually by allowing to cool, and appears again by heating.

Loss on drying <*5.01*> Not more than 17.0%.

Total ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.0%.

Cyperus Rhizome

コウブシ

Add the following next to Description:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cyperus 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 Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Cyperus Rhizome

コウブシ末

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Cyperus 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 Powdered Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Cyperus Rhizome does not show extremely lignified cells, except stone cells, and crystals.

Dioscorea Rhizome

サンヤク

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Dioscorea 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 Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Dioscorea Rhizome

サンヤク末

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Dioscorea 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 Powdered Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Fritillaria Bulb

バイモ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Fritillaria Bulb 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 Fritillaria Bulb according to Method 4, and perform the test (not more than 5 ppm).

Gastrodia Tuber

テンマ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Gastrodia Tuber 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 Gastrodia Tuber according to Method 4, and perform the test (not more than 5 ppm).

Gentian

ゲンチアナ

Add the following next to Identification:

Purity Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Gentian according to Method 4, and perform the test (not more than 5 ppm).

Powdered Gentian

ゲンチアナ末

Change the Purity to read:

Purity **(1)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Gentian according to Method 4, and perform the test (not more than 5 ppm).

(2) Foreign matter—Under a microscope <*5.01*>, stone cell and fiber are not observed.

Glehnia Root

ハマボウフウ

Add the following next to Description:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Glehnia 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 Glehnia Root according to Method 4, and perform the test (not more than 5 ppm).

Glycyrrhiza Extract

カンゾウエキス

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Glycyrrhiza Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Crude Glycyrrhiza Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Water-insoluble substances—Boil 5.0 g of pulverized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter the mixture through tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(3) Foreign matter—The filtrate obtained in (2) does not have a strong bitter taste.

(4) Starch—To about 1 g of pulverized Crude Glycyrrhiza Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Add the following:

Hangekobokuto Extract

半夏厚朴湯エキス

Hangekobokuto Extract contains not less than 2 mg and not more than 6 mg of magnolol, not less than 4 mg (for preparation prescribed 2 g of Perilla Herb) or not less than 6 mg (for preparation prescribed 3 g of Perilla Herb) of rosmarinic acid, and not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 1.3 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 per amount specified in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 6 g of Pinellia Tuber, 5 g of Poria Sclerotium, 3 g of Magnolia Bark, 2 g of Perilla Herb and 1 g of Ginger, or with 6 g of Pinellia Tuber, 5 g of Poria Sclerotium, 3 g of Magnolia Bark, 3 g of Perilla Herb and 1 g of Ginger, or with 6 g of Pinellia Tuber, 5 g of Poria Sclerotium, 3 g of Magnolia Bark, 2 g of Perilla Herb and 1.3 g of Ginger, or with 6 g of Pinellia Tuber, 5 g of Poria Sclerotium, 3 g of Magnolia Bark, 2 g of Perilla Herb and 1.5 g of Ginger.

Description Hangekobokuto Extract is a light brown to black-brown, powder or viscous extract. It has a characteristic odor and has a bitter and astringent taste first then pungent later.

Identification (1) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of magnolol 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 and hexane (1:1) 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 the several spots from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Magnolia Bark).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic 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 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, water and formic acid (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride 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 dark purple spot from the standard solution (Perilla Herb).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, 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 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 (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Ginger).

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).

Loss on drying <*2.41*> The dry extract: Not more than 11.0 % (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 14.0%, calculated on the dried basis.

Assay (1) Magnolol—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 (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of magnolol for component determination, previously dried in a desiccator (silica gel) for not less than 1 hour, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) 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 magnolol.

$$\text{Amount (mg) of magnolol} = W_S \times (A_T/A_S) \times (1/8)$$

W_S: Amount (mg) of magnolol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 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, acetonitrile and acetic

acid (100) (50:50:1).

Flow rate: 1.0 mL per minute. (the retention time of magnolol is about 15 minutes.)

System suitability—

System performance: Dissolve 1 mg each of magnolol for component determination and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol 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 magnolol is not more than 1.5%.

(2) Rosmarinic acid—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 (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of rosmarinic acid for component determination, dissolve in diluted methanol (7 in 10) 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, and determine the peak areas, A_T and A_S, of rosmarinic acid.

$$\text{Amount (mg) of rosmarinic acid} = W_S \times (A_T/A_S) \times (1/4)$$

W_S: Amount (mg) of rosmarinic acid for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 330 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 (800:200:1).

Flow rate: 1.0 mL per minute. (the retention time of rosmarinic acid is about 11 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 rosmarinic acid 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 rosmarinic acid is not more than 1.5%.

(3) [6]-Gingerol—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 (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 (7 in 10) 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%.

Containers and storage Containers—Tight containers.

Change to read:

Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 48 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 saikosaponin b_2, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 0.5 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 3 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Processed Ginger and 0.5 g of Cimicifuga Rhizome.

Description Hochuekkito Extract occurs as a light brown to black-brown powder or viscous extract. It has a slight odor, and a sweet and bitter taste.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and shake. Take the 1-butanol layer, evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 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, 1-propanol, water and acetic acid (100) (7:5:4:1) 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 (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 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 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 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 ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the sevelral spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, shake, and take the hexane layer. To the hexane layer add anhydrous sodium sulfate to dry, 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 appears around *R*f 0.4, which shows a greenish brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allowing to cool (Atractylodes Lancea Rhizome).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 40 mL of a solution of potassium hydroxide in methanol (1 in 50), shake for 15 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 30 mL of water to the residue, then add 20 mL of diethyl ether, shake, and take the water layer. To the water layer add 20 mL of 1-butanol, shake, and take the 1-butanol layer. To the 1-butanol layer add 20 mL of water, shake, take the 1-butanol layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside 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 5 μL each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water, 1-butanol and acetic acid (100) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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-brown spot from the standard solution (Astragalus Root).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide 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 chro-

matography. 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. 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 (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin 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 2 μL of the sample solution and 20 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (Citrus Unshiu Peel).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 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 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, 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: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Bupleurum Root).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of liquiritin 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 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 dilute 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 yellow-brown spot from the standard solution (Glycyrrhiza).

(9) For preparation prescribed Ginger—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 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 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 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Ginger).

(10) For preparation prescribed Processed Ginger—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 an 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 about 1 hour, separate the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of [6]-shogaol 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 60 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 cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Processed Ginger).

(11) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 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, acetone and water

(20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 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 yellow fluorescent spot from the standard solution (Cimicifuga Rhizome).

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).

Loss on drying <*2.41*> The dry extract: Not more than 11.5 % (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and 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 hesperidin.

$$\text{Amount (mg) of hesperidin} = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of hesperidin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

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

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

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL/min. (the retention time of hesperidin is about 15 minutes.)

System suitability—

System performance: Dissolve 1 mg each of hesperidin for component determination and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin 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 hespiridin is not more than 1.5%.

(2) Saikosaponin b_2—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 saikosaponin b_2 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. Pipet 10 mL of this solution, add 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 saikosaponin b_2.

$$\text{Amount (mg) of saikosaponin } b_2 = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of saikosaponin b_2 for component determination

Operating conditions—

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

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

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

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b_2 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 saikosaponin b_2 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 saikosaponin b_2 is not more than 1.5%.

(3) Glycyrrhizic acid—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 Glycyrrhizic Acid Reference Standard (separately determine the water), 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 glycyrrhizic acid.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of Glycyrrhizic Acid 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 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 diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid 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 glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Imperata Rhizome

ボウコン

Change the Purity to read:

Purity **(1)** Rootlet and scaly leaf—The amount of the rootlets and scaly leaves contained in Imperata Rhizome is not more than 3.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Imperata 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 0.40 g of pulverized Imperata 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 rootlets and scaly leaves is not more than 1.0%.

Ipecac

トコン

Add the following next to Identification:

Purity Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Ipecac according to Method 4, and perform the test (not more than 5 ppm).

Powdered Ipecac

トコン末

Change the Purity to read:

Purity **(1)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Ipecac according to Method 4, and perform the test (not more than 5 ppm).

(2) Foreign matter—Under a microscope <*5.01*>, groups of stone cells and thick-walled fibers are not observed.

Japanese Gentian

リュウタン

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Japanese Gentian 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 Gentian according to Method 4, and perform the test (not more than 5 ppm).

Powdered Japanese Gentian

リュウタン末

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Japanese Gentian 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 Gentian according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Japanese Gentian usually reveals no stone cells and fibers. No starch grains; if any, very few.

Japanese Valerian

カノコソウ

Add the following next to Description:

Purity 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

Add the following next to Description:

Purity 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 to read:

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephedra 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 less than 42 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peony Root) of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), 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.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 8 g of Pueraria Root, 4 g of Ephedra Herb, 4 g of Jujube, 3 g of Cinnamon Bark, 3 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 4 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 3 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 3 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 2 g of Ginger.

Description Kakkonto Extract occurs as a light brown to black-brown powder or viscous extract. It has a characteristic odor, and a sweet first, then hot, and slightly bitter 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 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under 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. 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 (Pueraria Root).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ephedrine hydrochloride 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 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin 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 red-purple spot from the standard solution (Ephedra Herb).

(3) 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 the hexane layer, and use the layer 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 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. 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 (Cinnamon Bark).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 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).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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 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 dilute 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 yellow-brown spot from the standard solution (Glycyrrhiza).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the 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, 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 blue-green spot from the standard solution (Ginger).

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).

Loss on drying <*2.41*> The dry extract: Not more than 10.0 % (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 10.0%, calculated on the dried basis.

Assay **(1)** Total alkaloids (ephedrine and pseudoephedrine)—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 ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) 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 the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine with the sample solution, and the peak area, A_S, of ephedrine with the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]

$$= W_S \times \{(A_{TE} + A_{TP})/A_S\} \times 0.819 \times (1/10)$$

W_S: Amount (mg) of ephedrine hydrochloride for assay

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 40°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL/min. (the retention time of ephedrine is about 27 minutes.)

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride 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, pseudoephedrine and ephedrine 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 ephedrine 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, and filter.

Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with water to make exactly 20 mL of eluate, and use this 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. Pipet 5 mL of this solution, add diluted methanol (1 in 2) 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 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/min. (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) Glycyrrhizic acid—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 Glycyrrhizic Acid Reference Standard (separately determine the water) 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 glycyrrhizic acid.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of Glycyrrhizic Acid 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 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 diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid 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 glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kamishoyosan Extract

加味逍遙散エキス

Change to read:

Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 16 mg and not more than 48 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.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 2 g of Glycyrrhiza, 1 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyr-

rhiza, 1 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyrrhiza, 1.5 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Mentha Herb.

Description Kamishoyosan Extract occurs as a yellow-brown to black-brown powder or viscous extract. It has slightly a characteristic odor, and a sweet, slightly hot, then bitter taste.

Identification **(1)** 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 (*Z*)-ligustilide 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 ethyl acetate and hexane (1:1) 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 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 (Japanese Angelica 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 albiflorin 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, methanol and ammonia solution (28) (6:3:2) 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 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 orange fluorescent spot from the standard solution (Peony Root).

(3) For preparation prescribed Atractylodes Rhizome—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 atractylenolide III 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-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 red spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous 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 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 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 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, 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: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Bupleurum Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of peonol 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 hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one 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).

(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, dissolve 1 mg of geniposide 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, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehide-sulfric 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 (Gardenia Fruit).

(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, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of 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 (Glycyrrhiza).

(9) 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, centrifuge, and use the supernatant liquid 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 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 4-dimethylaminobenzaldehyde TS for spraying 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 blue-green spot from the standard solution (Ginger).

(10) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 0.2 g of pulverized Mentha Herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, 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 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 (10:1:1) 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 red-purple spot (around *R*f 0.6) from the standard solution (Mentha Herb).

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).

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 10.0%, calculated on the dried basis.

Assay **(1)** 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/min. (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%.

(2) Geniposide—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 geniposide for component determination, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) 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 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 geniposide.

$$\text{Amount (mg) of geniposide} = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of geniposide for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

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

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

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL/min. (the retention time of geniposide 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 number of theoretical plates and the symmetry factor of the peak of geniposide 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 geniposide is not more than 1.5%.

(3) Glycyrrhizic acid—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 Glycyrrhizic Acid Reference Standard (separately determine the water), 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 glycyrrhizic acid.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of Glycyrrhizic Acid 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 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 diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid 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 glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Add the following:

Keishibukuryogan Extract

桂枝茯苓丸エキス

Keishibukuryogan Extract contains not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 3 g of Cinnamon Bark) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 4 g of Cinnamon Bark) of (*E*)-cinnamic acid, not less than 30 mg and not more than 90 mg (for preparation prescribed 3 g each of Moutan Bark and Peony Root) or not less than 40 mg and not more than 120 mg (for preparation prescribed 4 g each of Moutan Bark and Peony Root) of peoniflorin ($C_{23}H_{28}O_{11}$:

480.46), and not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peach Kernel) or not less than 28 mg and not more than 84 mg (for preparation prescribed 4 g of Peach Kernel) of amygdalin per amount specified in the Method of preparation.

Method of preparation Prepare a dry extract or viscous extract as directed under Extracts, with 4 g of Cinnamon Bark, 4 g of Poria Sclerotium, 4 g of Moutan Bark, 4 g of Peach Kernel and 4 g of Peony Root, or prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive, prepared as directed under Extracts with 3 g of Cinnamon Bark, 3 g of Poria Sclerotium, 3 g of Moutan Bark, 3 g of Peach Kernel and 3 g of Peony Root.

Description Keishibukuryogan Extract is a light brown to black-brown, powder or viscous extract. It has a characteristic odor and has a taste slightly sweet first then bitter later.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic 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 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) 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 the several spots from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of peonol 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 hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one 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).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin 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 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 4-methoxybenzaldehyde-sulfuric acid TS 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 green-brown spot from the standard solution (Peach Kernel).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin 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 ammonia water (28) (6:3:2) 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 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 orange fluorescent spot from the standard solution (Peony 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).

Loss on drying <*2.41*> The dry extract: Not more than 10.0 % (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 10.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

Assay **(1)** (*E*)-Cinnamic acid—Conduct this procedure without exposure to light, using light-resistant vessels. 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 (*E*)-cinnamic acid 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. Pipet 10 mL of this solution, add 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 (*E*)-cinnamic acid.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of (*E*)-cinnamic acid for component determination

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 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute. (the retention time of (*E*)-cinnamic acid 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 (*E*)-cinnamic acid 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 (*E*)-cinnamic acid is not more than 1.5%.

(2) Paeoniflorin—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 Paeoniflorin Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) 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 paeoniflorin.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = W_S \times (A_T/A_S)$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

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

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute. (the retention time of paeoniflorin is about 9 minutes.)

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Amygdalin—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 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 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 amygdalin.

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

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 octadecylsilanized 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%.

Containers and storage Containers—Tight containers.

Add the following:

Leonurus Herb

Leonuri Herba

ヤクモソウ

Leonurus Herb is the aerial part of *Leonurus japonicus* Houttuyn or *Leonurus sibiricus* Linné (*Labiatae*), collected during the flowering season.

Description Stem, leaves, and flowers usually cross sectioned, stems squre, 0.2 to 3 cm in diameter, yellow-green to green-brown in color, covered densely with white short hairs; the pith white, a great parts of central of sections. Light in texture. Leaves opposite, petiolated, 3-dissected to 3-incised, each lobes split pinnately, and end lobes reveals linear-lanceolate, acute or acuminate, the upper surface light green, the lower surface bristle with white short hairs, grayish green. Flower, verticillate; sepal, tubular, and the upper end acerate with five lobes; light green to light green-brown in color, corolla labiate, light red-purple to light brown.

Odor, slightly; taste, slightly bitter, astringent.

Under a microscope <*5.01*>, a transverse section of stem reveals four ridge, a parts of the ridge of *Leonurus sibiricus* Linné protruding knobby. Epidermis, observed non-glandular hairs from 1 to 3 cells, glandular hairs with head of 1 to 4 celled or glandular scale with 8 cells. Each ridge parts, beneath epidermis, collenchyma developed, development of xylem fibres remarkably. Cortex composed of several layers parenchymatous cells. Collateral vascular bundle arranged in a circle. Phloem fibres observed at the outer portion of phloem. Parenchymatous cells of cortex and pith observe needle crystals or plate-like crystals of calcium oxalate.

Identification To 1 g of pulverized Leonurus Herb add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under 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 water and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying followed by immediate spraying of sodium nitrite TS on the plate: a grayish brown spot appears at around *R*f 0.5, which color fades soon and then disappears after air-drying the plate.

Loss on drying <*5.01*> Not more than 12.0%.

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 12.0%.

Add the following:

Lilium Bulb

Lilii Bulbus

ビャクゴウ

Lilium Bulb is the scaly leaves of *Lilium lancifolium* Thunberg, *Lilium brownii* F.E.Brown var. *colchesteri* Wilson, *Lilium brownii* F.E.Brown or *Lilium pumilum* De Candolle (*Liliaceae*), usually with the application of steaming.

Description Lilium Bulb reveals oblong with narrowed apex, lanceolate, or narrowly triangular boat-shaped, translucent, 1.3 to 6 cm in length, 0.5 to 2.0 cm in diameter, externally milky white to light yellowish brown occasionally purplish in color, nearly smooth; central portion somewhat thickend, circumferential portion thin, slightly waved, occasionally rolled inside; usually several lines of vascular bundles longitudinally in parallel are seen through parenchyma; hard in texture, easy to break; fractured surface horny and flat.

Odorless; taste, slightly acid and bitter.

Under a microscope <*5.01*>, the surface reveals epidermal cells rectangular to almost square, stomata nearly circular, the cells adjacent to stomata mostly 4 in number. Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of epidermal cells covered with smooth cuticle; beneath epidermis circular to quadrangular parenchymatous cells distributed evenly, palisade tissue not observed; in parenchyma of mesophyll collateral vascular bundles extended from adaxial side to abaxial side of scaly leaves are arranged almost in a transverse line; starch grains contained in parenchymatous cells, usually gelatinized.

Identification To 3 g of pulverized Lilium Bulb add 10 mL of 1-butanol, shake, add 10 mL of water, shake for 30 minutes, and centrifuge. Evaporate the supernatant liquid under reduced pressure, add 1 mL of methanol to the residue, shake gently, and use the supernatant liquid so obtained as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at around *R*f 0.3. When examine these spots under ultraviolet light (main wavelength: 365 nm) after spraying with sodium carbonate

TS, they appear as blue-purple fluorescent spots.

Loss on drying ⟨*5.01*⟩ Not more than 16.0%.

Total ash ⟨*5.01*⟩ Not more than 4.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 8.0%.

Lindera Root

ウヤク

Add the following next to Identification:

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Lindera 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 Lindera Root according to Method 4, and perform the test (not more than 5 ppm).

Lithospermum Root

シコン

Add the following next to Identification:

Purity 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).

Lycium Bark

ジコッピ

Add the following next to Identification:

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Lycium Bark 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 Lycium Bark according to Method 4, and perform the test (not more than 5 ppm).

Magnolia Bark

コウボク

Change the Component determination to read:

Component determination Weigh accurately about 0.5 g of pulverized Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for component determination in a desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) 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 magnolol in each solution.

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

W_S: Amount (mg) of magnolol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for component determination and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol 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 magnolol is not more than 1.5%.

Powdered Magnolia Bark

コウボク末

Change the Component determination to read:

Component determination Weigh accurately about 0.5 g of Powdered Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for component determination in a desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) 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 magnolol in each solution.

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

W_S: Amount (mg) of magnolol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for component determination and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol 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 magnolol is not more than 1.5%.

Mulberry Bark

ソウハクヒ

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Mulberry Bark 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 Mulberry Bark according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of the root xylem and other foreign matter is not more than 1.0%.

Notopterygium Rhizome

キョウカツ

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Notopterygium 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 Notopterygium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Nuphar Rhizome

センコツ

Change the Purity to read:

Purity **(1)** Petiole—The amount of its petioles contained in Nuphar Rhizome does not exceed 3.0%.

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Nuphar Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of foreign matter other than petiole is not more than 1.0%.

Nux Vomica Extract

ホミカエキス

Add the following next to Identification:

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Nux Vomica Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Panax Japonicus Rhizome

チクセツニンジン

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Panax Japonicus 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 Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Panax Japonicus Rhizome

チクセツニンジン末

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Panax Japonicus 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 Powdered Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Peach Kernel

トウニン

Change the Identification to read:

Identification To 1.0 g of ground Peach Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin 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, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon 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-brown spot from the standard solution.

Powdered Peach Kernel

トウニン末

Change the Identification to read:

Identification **(1)** Grind Powdered Peach Kernel with water: the odor of benzaldehyde is perceptible.

(2) To 1.0 g of Powdered Peach Kernel add 10 mL of methanol, and immediately heat under a reflux condenser on a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin 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, methanol and water (20:5:4) to a distance of about 10 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying 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-brown spot from the standard solution.

Perilla Herb

ソヨウ

Change the Purity (1) to read:

Purity **(1)** Stem—Perilla Herb does not contain its stems equal to or greater than 3 mm in diameter.

Add the following:

Peucedanum Root

Peucedani Radix

ゼンコ

Peucedanum Root is the root of **1)** *Peucedanum praeruptorum* Dunn or **2)** *Angelica decursiva* Franchet et Savatier (*Peucedanum decursivum* Maximowicz) (*Umbelliferae*).

Description **1)** *Peucedanum praeruptorum* Dunn

Slender obconical to cylindrical root, occasionally dichotomized at the lower part 3 to 15 cm in length, 0.8 to 1.8 cm in diameter at the crown; externally light brown to dark brown; ring-node-like wrinkles numerous at the crown, sometimes with hair-like remains of petioles; the root having somewhat deep longitudinal wrinkles and scars of cutting off of lateral roots; cross section surface light brown to whitish in color; brittle in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of a cork layer, inner tangential walls of some cork cells thickened; collenchyma just inside of the cork layer; in cortex numerous oil canals scattered and intercellular air spaces observed; occasionally phloem fibers observed at the terminal portion of phloem; vessels and scattered oil canals in xylem; starch grains in parenchyma, 2 to 10 several-compound grains.

2) *Angelica decursiva* Franchet et Savatier

Similar to 1), but without hair-like remains of petioles at the crown.

Under a microscope <*5.01*>, a transverse section reveals, similar to 1), but cell wall of cork cells not thickened, phloem fibers not observed at the terminal portion of phloem, nor oil canals observed in xylem.

Identification **(1)** *Peucedanum praeruptorum* Dunn—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (±)-praeruptorin 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 mix-

ture of diethyl ether and hexane (3:1) 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 the several spots from the sample solution has the same color tone and *R*f value with the blue-purple fluorescent spot from the standard solution.

(2) *Angelica decursiva* Franchet et Savatier—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nodakenin 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, methanol and water (12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple fluorescent spot from the standard solution.

Loss on drying <*5.01*> Not more than 13.0%.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 20.0%.

Platycodon Fluidextract

キキョウ流エキス

Change the Purity to read:

Purity (1) Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Platycodon Fluidextract as directed in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Starch—Mix 1 mL of Platycodon Fluidextract with 4 mL of water, and add 1 drop of dilute iodine TS: no purple or blue color develops.

Polygala Root

オンジ

Change the Purity to read:

Purity (1) Stem—The amount of the stems contained in Polygala Root does not exceed 10.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polygala 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).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Polygala Root 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%.

(5) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Powdered Polygala Root

オンジ末

Change the Purity to read:

Purity (1) Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Polygala 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 Powdered Polygala Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Polygala Root does not show stone cells or starch grains.

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Polygonum Root

カシュウ

Add the following next to Identification:

Purity (1) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polygonum 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 Polygonum Root according to Method 4, and perform the test (not more than 5 ppm).

Polyporus Sclerotium

チョレイ

Add the following next to Identification:

Purity (1) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polyporus Sclerotium 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 Polyporus Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Powdered Polyporus Sclerotium

チョレイ末

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Polyporus Sclerotium 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 Polyporus Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Processed Aconite Root

ブシ

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Processed Aconite 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 0.4 g of pulverized Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and 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. Perform the test with exactly 20 μL each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA}, jesaconitine, H_{TJ} and H_{SJ}, hypaconitine, H_{TH} and H_{SH}, and mesaconitine, H_{TM} and H_{SM}, respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 60 μg, 60 μg, 280 μg and 140 μg, respectively, and the total amount of them is not more than 450 μg.

Amount (μg) of aconitine ($C_{34}H_{47}NO_{11}$)
$= (C_{SA}/W) \times (H_{TA}/H_{SA}) \times 10$

Amount (μg) of jesaconitine ($C_{35}H_{49}NO_{12}$)
$= (C_{SJ}/W) \times (H_{TJ}/H_{SJ}) \times 10$

Amount (μg) of hypaconitine ($C_{33}H_{45}NO_{10}$)
$= (C_{SH}/W) \times (H_{TH}/H_{SH}) \times 10$

Amount (μg) of mesaconitine ($C_{33}H_{45}NO_{11}$)
$= (C_{SM}/W) \times (H_{TM}/H_{SM}) \times 10$

C_{SA}: Concentration (μg/mL) of aconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SJ}: Concentration (μg/mL) of jesaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SH}: Concentration (μg/mL) of hypaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SM}: Concentration (μg/mL) of mesaconitine for purity in aconitum diester alkaloids standard solution for purity

W: Amount (g) of sample, calculated on the dried basis

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 solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust the flow rate so that 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: To 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 10 mL. When the test is repeated 6 times with 20 μL of this 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%.

Powdered Processed Aconite Root

ブシ末

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of

Powdered Processed Aconite 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 0.4 g of Powdered Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process two times. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and 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. Perform the test with exactly 20 μL each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA}, jesaconitine, H_{TJ} and H_{SJ}, hypaconitine, H_{TH} and H_{SH}, and mesaconitine, H_{TM} and H_{SM}, respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 55 μg, 40 μg, 55 μg and 120 μg, respectively, and the total amount of them is not more than 230 μg.

$$\text{Amount } (\mu g) \text{ of aconitine } (C_{34}H_{47}NO_{11}) = (C_{SA}/W) \times (H_{TA}/H_{SA}) \times 10$$

$$\text{Amount } (\mu g) \text{ of jesaconitine } (C_{35}H_{49}NO_{12}) = (C_{SJ}/W) \times (H_{TJ}/H_{SJ}) \times 10$$

$$\text{Amount } (\mu g) \text{ of hypaconitine } (C_{33}H_{45}NO_{10}) = (C_{SH}/W) \times (H_{TH}/H_{SH}) \times 10$$

$$\text{Amount } (\mu g) \text{ of mesaconitine } (C_{33}H_{45}NO_{11}) = (C_{SM}/W) \times (H_{TM}/H_{SM}) \times 10$$

C_{SA}: Concentration (μg/mL) of aconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SJ}: Concentration (μg/mL) of jesaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SH}: Concentration (μg/mL) of hypaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SM}: Concentration (μg/mL) of mesaconitine for purity in aconitum diester alkaloids standard solution for purity

W: Amount (g) of the sample, calculated on the dried basis

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 solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust the flow rate so that 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: To 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 10 mL. When the test is repeated 6 times with 20 μL of this 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%.

Processed Ginger

カンキョウ

Add the following next to Identification:

Purity Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Processed Ginger according to Method 4, and perform the test (not more than 5 ppm).

Rehmannia Root

ジオウ

Change the Description to read:

Description Thin and long, usually, fusiform root, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter, often broken or markedly deformed in shape; externally yellow-brown to blackish brown, with deep, longitudinal wrinkles and constrictions; soft in texture and mucous; cross section yellow-brown to blackish brown, and cortex darker than xylem in color; pith hardly observable.

Odor, characteristic; taste, slightly sweet at first, followed by a slight bitterness.

Under a microscope <*5.01*>, a transverse section reveals 7 to 15 layers of cork; cortex composed entirely of parenchyma cells; outer region of cortex with scattered cells contain-

ing brown secretes; xylem practically filled with parenchyma; vessels radially lined, mainly reticulate vessels.

Change to read:

Ryokeijutsukanto Extract

苓桂朮甘湯エキス

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, and not less than 21 mg and not more than 63 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dry or viscous extract as directed under Extracts, with 6 g of Poria Sclerotium, 4 g of Cinnamon Bark, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome and 2 g of Glycyrrhiza.

Description Ryokeijutsukanto Extract occurs as a brown to black-brown powder or viscous extract. It has an odor, and a sweet first then bitter taste.

Identification **(1)** To 1.0 g of dry extract (3.0 g for viscous extract) of Ryokeijutsukanto 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 (*E*)-cinnamic 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 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) 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 the several spots from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of dry extract (3.0 g for viscous extract) of Ryokeijutsukanto 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 dilute sulfuric acid on the plat, 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 dry extract (6.0 g for viscous extract) of Ryokeijutsukanto 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 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 dry extract (3.0 g for viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin 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 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 dilute 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 yellow-brown spot from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) of Ryokeijutsukanto 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 dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) of Ryokeijutsukanto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> Dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

Viscous extract: Not more than 66.7% (1 g 105°C, 5 hours).

Total ash <*5.01*> Not more than 8.0%, calculated on the

dried basis.

Assay **(1)** (*E*)-Cinnamic acid—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.5 g of dry extract (for viscous extract an amount equivalent to about 0.5 g as dried substance) of Ryokeijutsukanto 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 (*E*)-cinnamic acid for component determination, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add 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 (*E*)-cinnamic acid.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of (*E*)-cinnamic acid for component determination

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 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute. (the retention time of (*E*)-cinnamic acid 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 (*E*)-cinnamic acid 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 (*E*)-cinnamic acid is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of dry extract (for viscous extract an amount equivalent to about 0.5 g as dried substance) of Ryokeijutsukanto 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 Glycyrrhizic Acid Reference Standard (separately determin the water), 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 glycyrrhizic acid.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of Glycyrrhizic Acid 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 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 diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute. (the retention time of glycyrrhizic acid 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 glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Saposhnikovia Root

ボウフウ

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Saposhnikovia 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 Saposhnikovia Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of stems and other foreign matter is not more than 2.0%.

Saussurea Root

モッコウ

Change the Purity to read:

Purity **(1)** Arsenic <*1.11*>—Prepare the test solution with

0.40 g of pulverized Saussurea Root according to Method 4, and perform the test (not more than 5 ppm).

(2) Foreign matter—Add iodine TS dropwise to a transverse section: no blue-purple color develops.

Scopolia Extract

ロートエキス

Add the following next to Identification:

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Scopolia Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Scopolia Rhizome

ロートコン

Add the following next to Identification:

Purity 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).

Scutellaria Root

オウゴン

Change the Assay to read:

Assay Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of the extract, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (separately determine the water), and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (7 in 10) 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 the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = W_S \times (A_T/A_S) \times 5$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 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 diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

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

System suitability—

System performance: Dissolve 1 mg of Baicalin Reference Standard and 2 mg of methyl parahydroxybenzoate in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Powdered Scutellaria Root

オウゴン末

Change the Assay to read:

Assay Weigh accurately about 0.5 g of Powdered Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of the extract, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (separately determine the water), and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (7 in 10) 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 the peak areas, A_T and A_S, of baicalin in each solution.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$) = $W_S \times (A_T/A_S) \times 5$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 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 diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

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

System suitability—

System performance: Dissolve 1 mg of Baicalin Reference Standard and 2 mg of methyl parahydroxybenzoate in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Senega

セネガ

Change the Purity to read:

Purity **(1)** Stem—The amount of stems contained in Senega is not more than 2.0%.

(2) 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).

(3) Foreign matter <*5.01*>—The amount of foreign matter other than stems contained in Senega is not more than 1.0 %.

Powdered Senega

セネガ末

Change the Purity to read:

Purity **(1)** 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).

(2) Foreign matter—Under a microscope <*5.01*>, stone cells, starch grains or crystals of calcium oxalate are not observable.

Smilax Rhizome

サンキライ

Add the following next to Description:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Smilax 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 Smilax Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Smilax Rhizome

サンキライ末

Change the Purity to read:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Smilax 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 Powdered Smilax Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Smilax Rhizome does not show a large quantity of stone cells or thick-walled fibers.

Sophora Root

クジン

Change the Purity to read:

Purity **(1)** Stem—The amount of its stems contained in Sophora Root does not exceed 10.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Sophora 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).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Sophora Root 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 stems is not more than 1.0%.

Powdered Sophora Root

クジン末

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Sophora 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 Powdered Sophora Root according to Method 4, and perform the test (not more than 5 ppm).

Turmeric

ウコン

Add the following next to Identification:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Turmeric 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 Turmeric according to Method 4, and perform the test (not more than 5 ppm).

Add the following:

Powdered Turmeric

Curcumae Rhizoma Purveratum

ウコン末

Powdered Turmeric is the powder of Turmeric.

Description Powdered Turmeric occurs as a yellow-brown to dark yellow-brown powder. It has a characteristic odor and a bitter, stimulant taste, and colors the saliva yellow on chewing.

Under a microscope <*5.01*>, all elements are yellow in color; it reveals parenchymatous cells containing mainly masses of gelatinized starch or yellow substances, also fragments of scalariform vessels; fragments of cork layers, epidermis, thick-walled xylem parenchymatous cells, and non-glandular hairs are occasionally observed.

Identification 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 of about 10 cm, and air-dry the plate: a yellow spot appears at around *R*f 0.4.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Turmeric 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 Turmeric according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 17.0% (6 hours).

Total ash <*5.01*> Not more than 7.5%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 9.0%.

Uva Ursi Fluidextract

ウワウルシ流エキス

Add the following next to Identification:

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Uva Ursi Fluidextract as direct in the Fluidextracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

Zedoary

ガジュツ

Add the following next to Description:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Zedoary according to Method 3, 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 0.40 g of pulverized Zedoary according to Method 4, and perform the test (not more than 5 ppm).

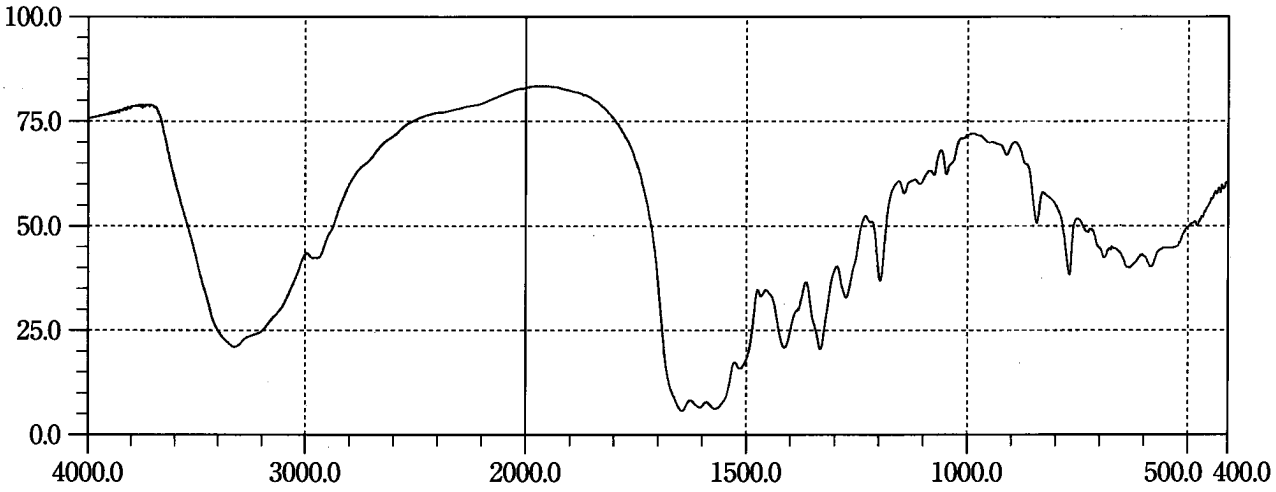
Delete the following Infrared Reference Spectra:

Fosfestrol

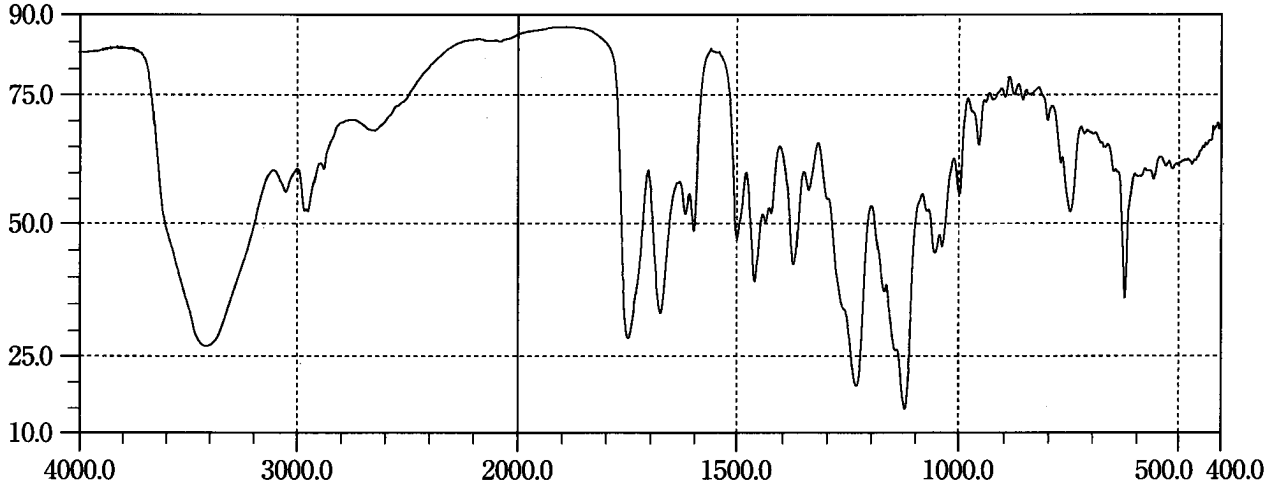
Sulfinpyrazone

Change to read the following 2 spectra:

Calcium Folinate

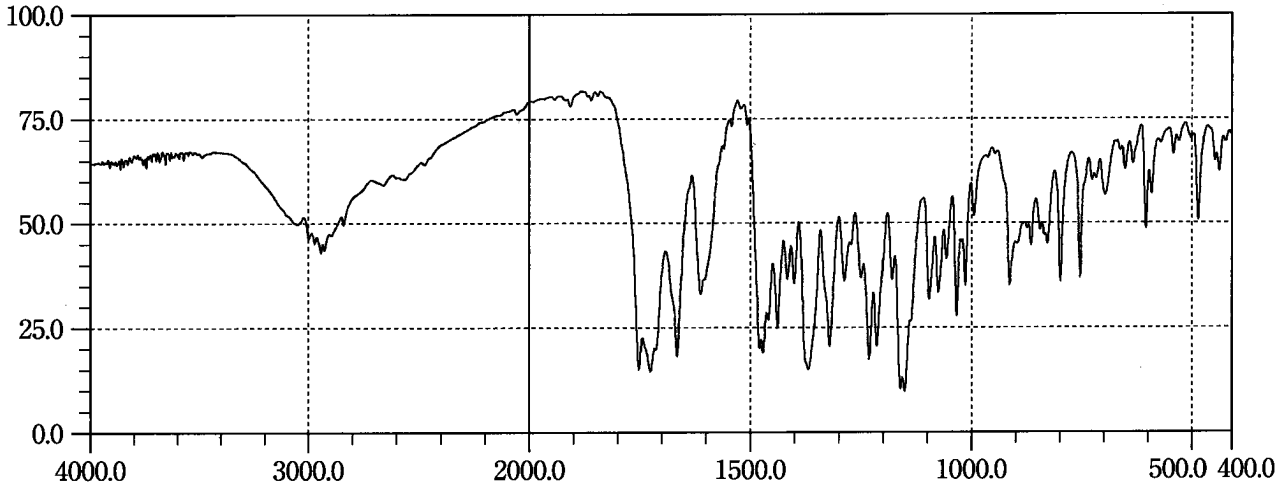


Vincristine Sulfate

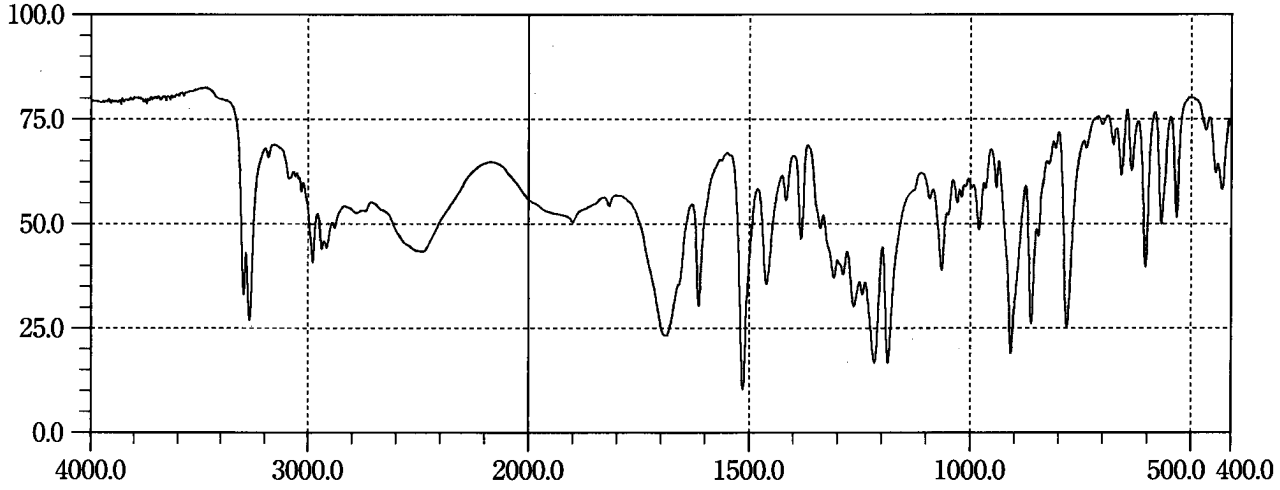


Add the following 42 spectra:

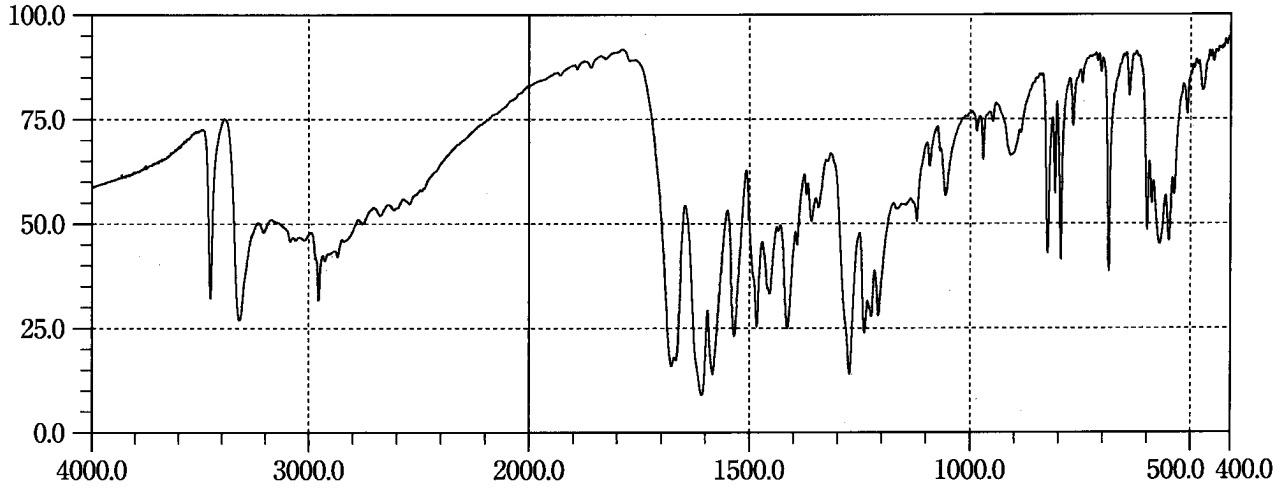
Acemetacin



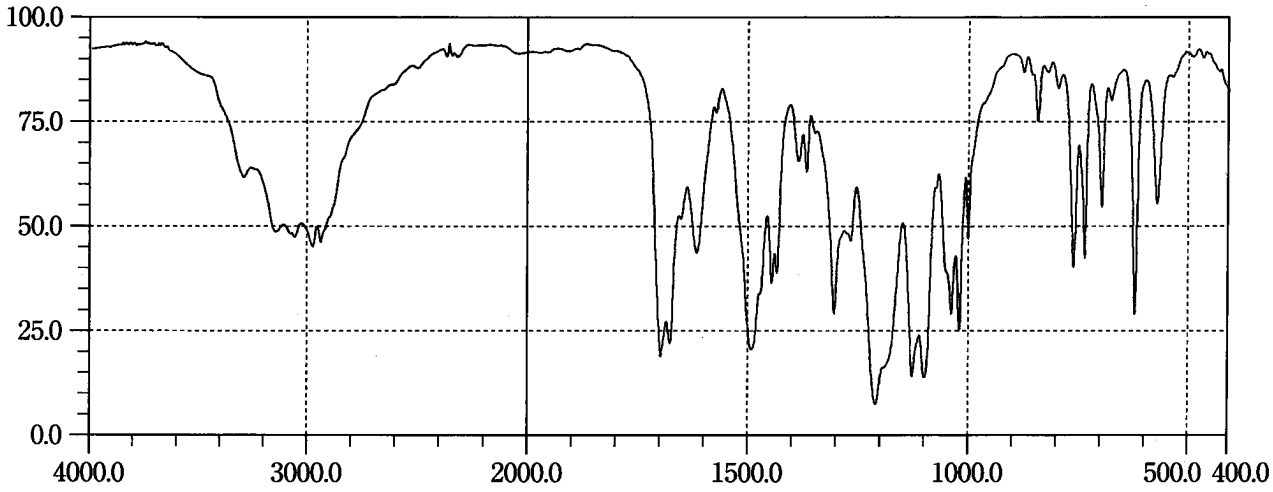
Alminoprofen



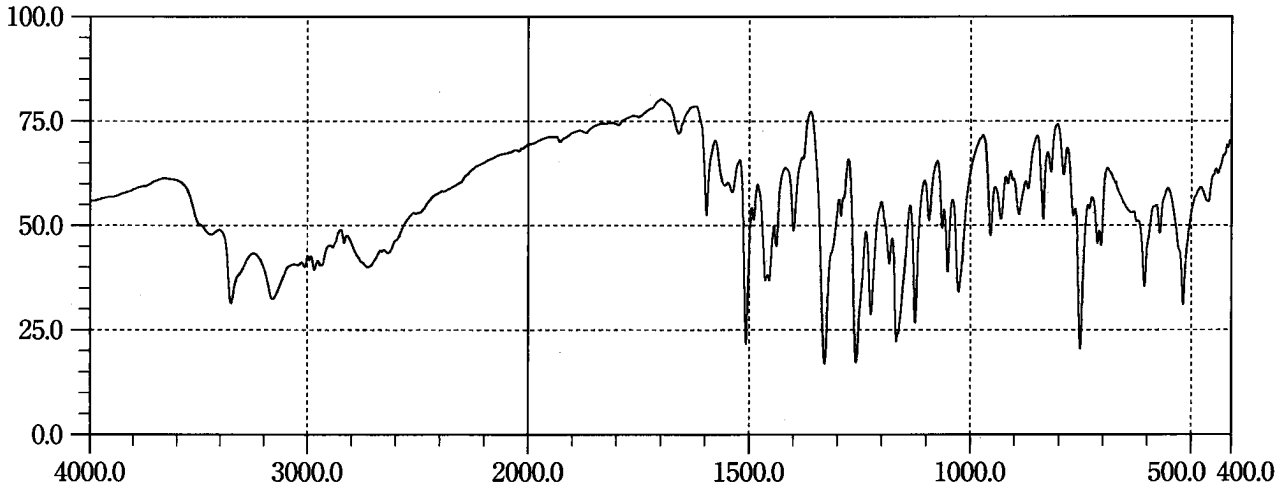
Amlexanox



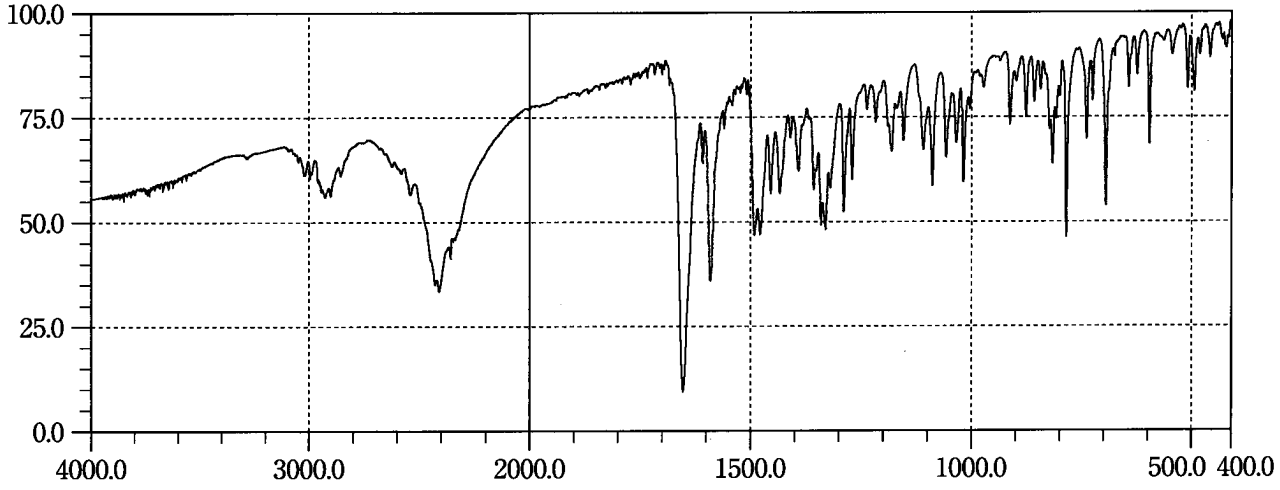
Amlodipine Besilate



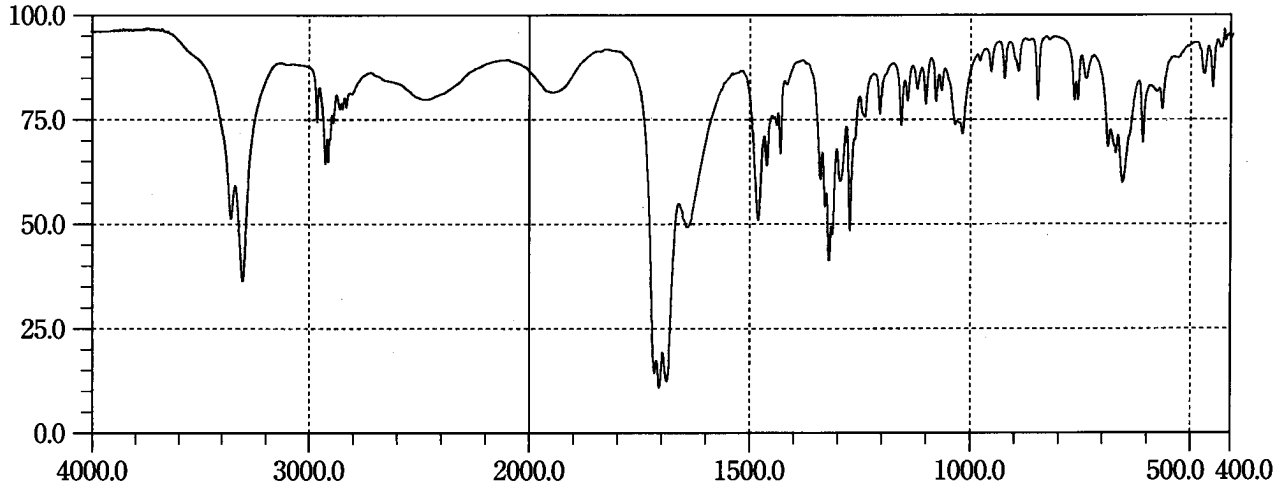
Amosulalol Hydrochloride



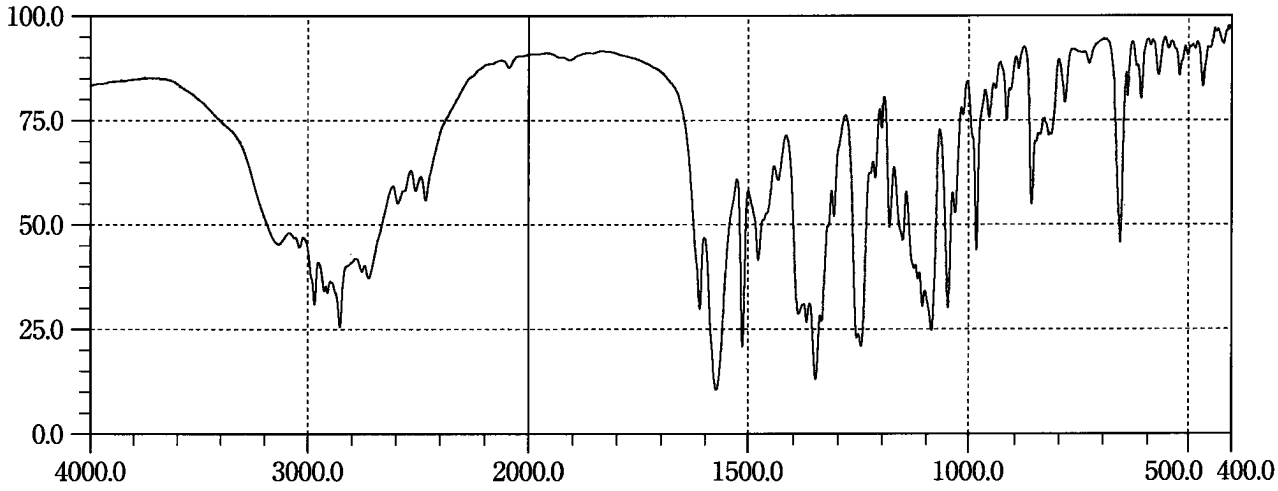
Azelastine Hydrochloride



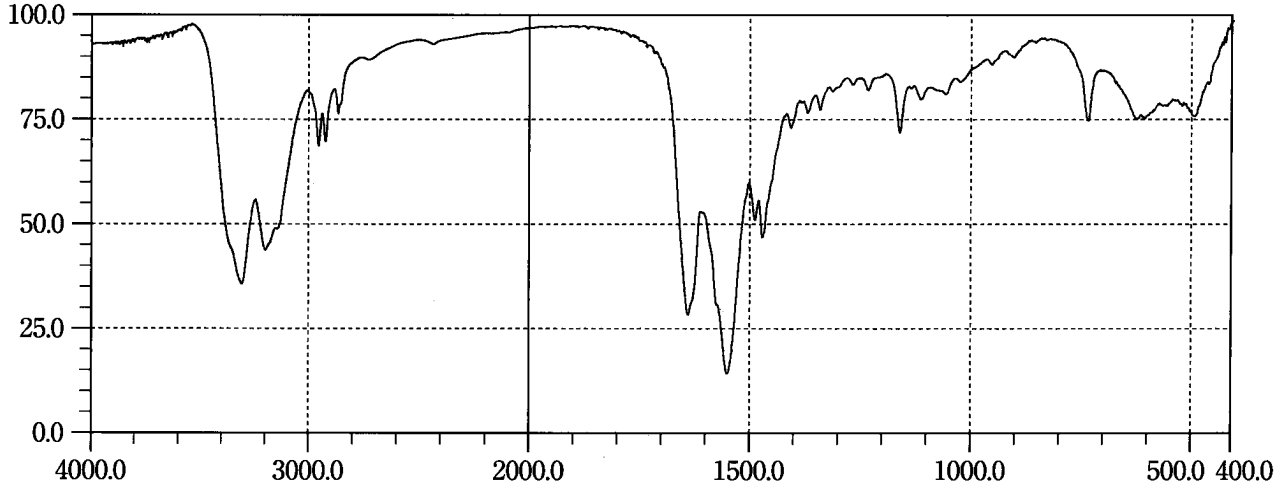
Biotin



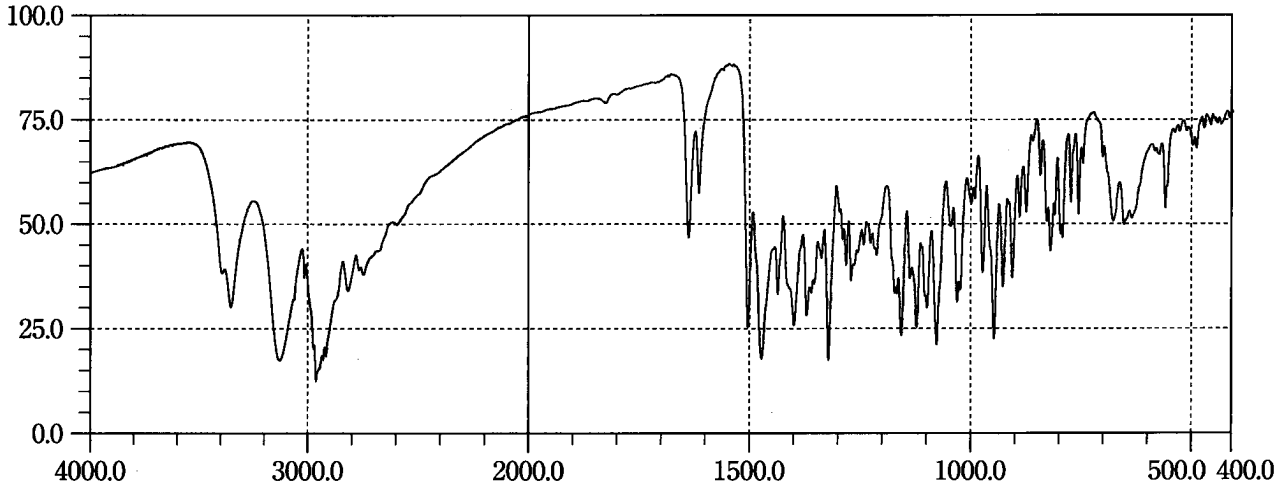
Bisoprolol Fumarate



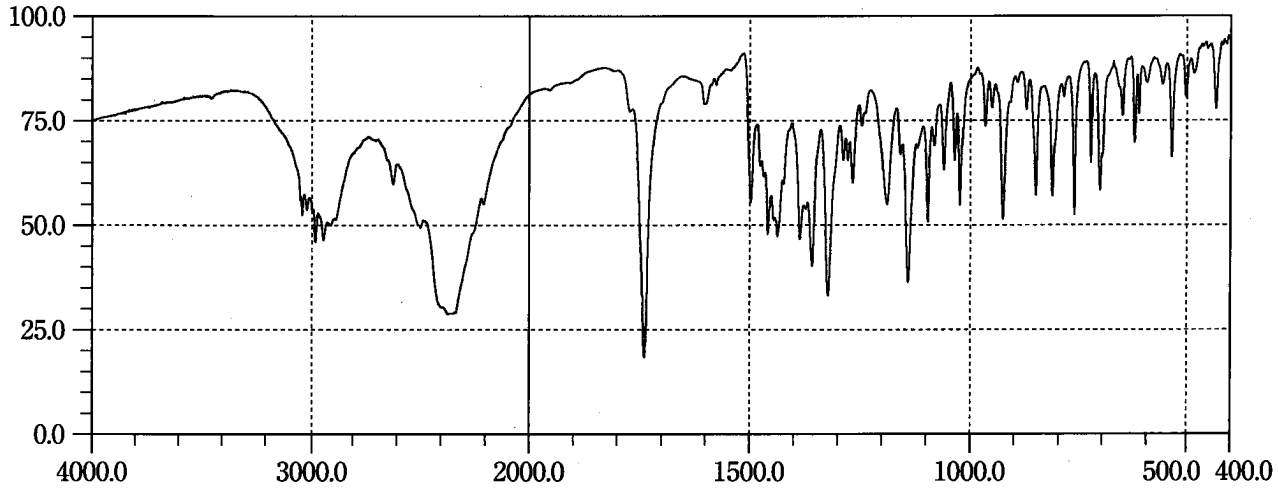
Buformin Hydrochloride



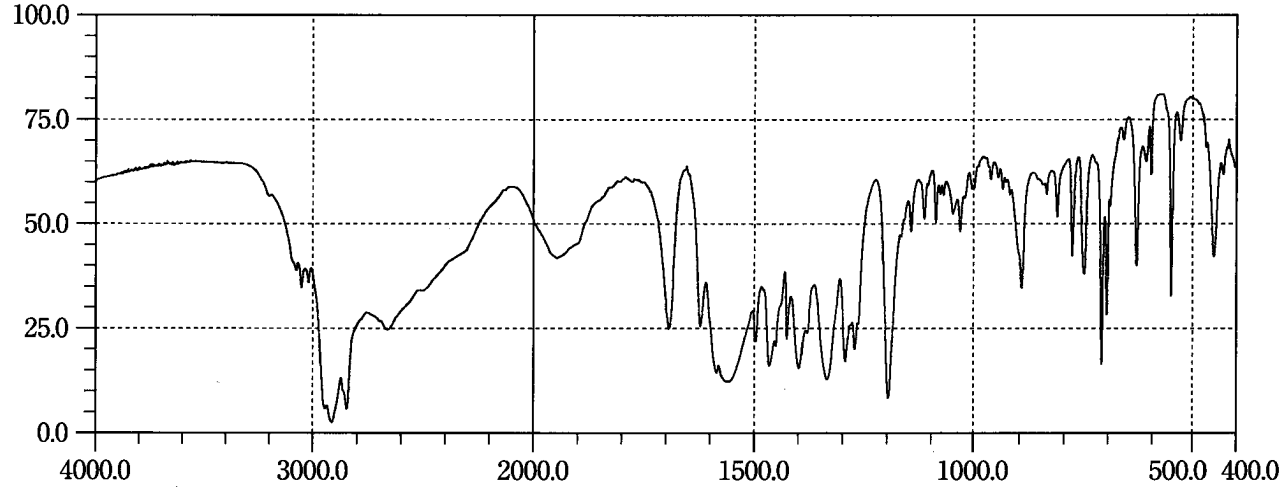
Buprenorphine Hydrochloride



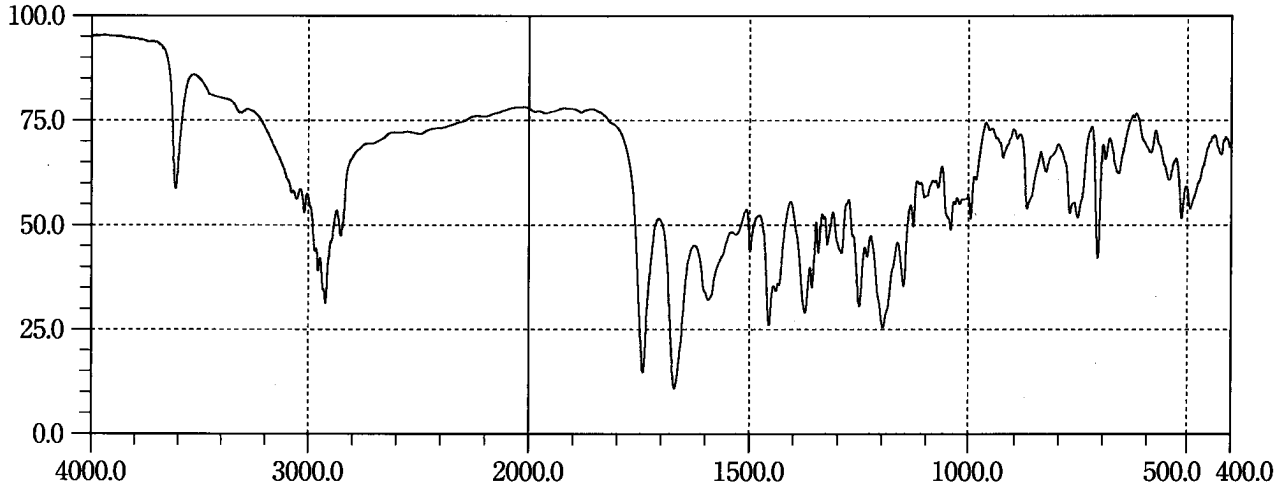
Cetirizine Hydrochloride



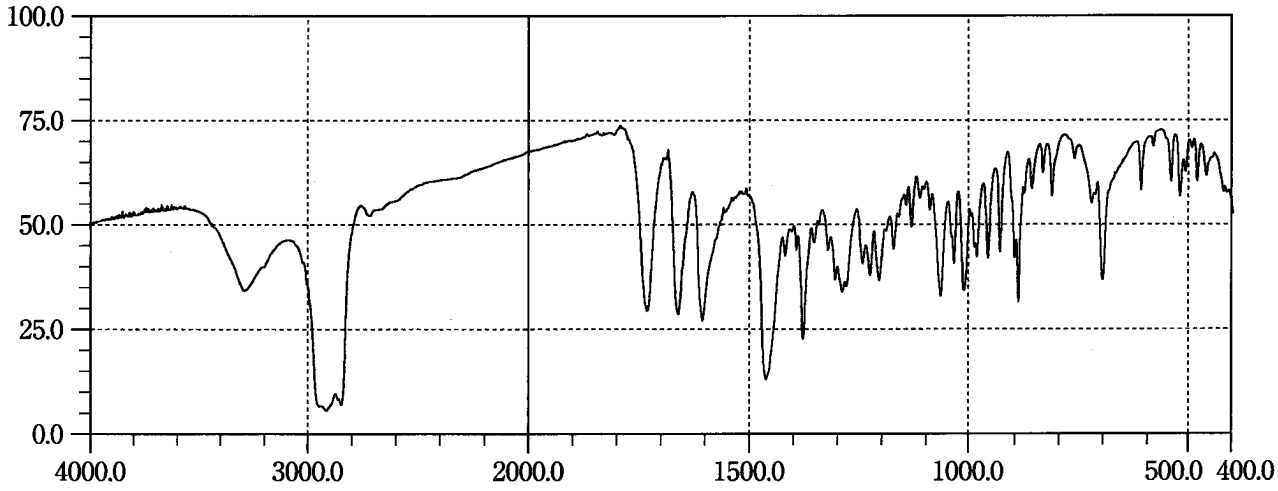
Cibenzoline Succinate



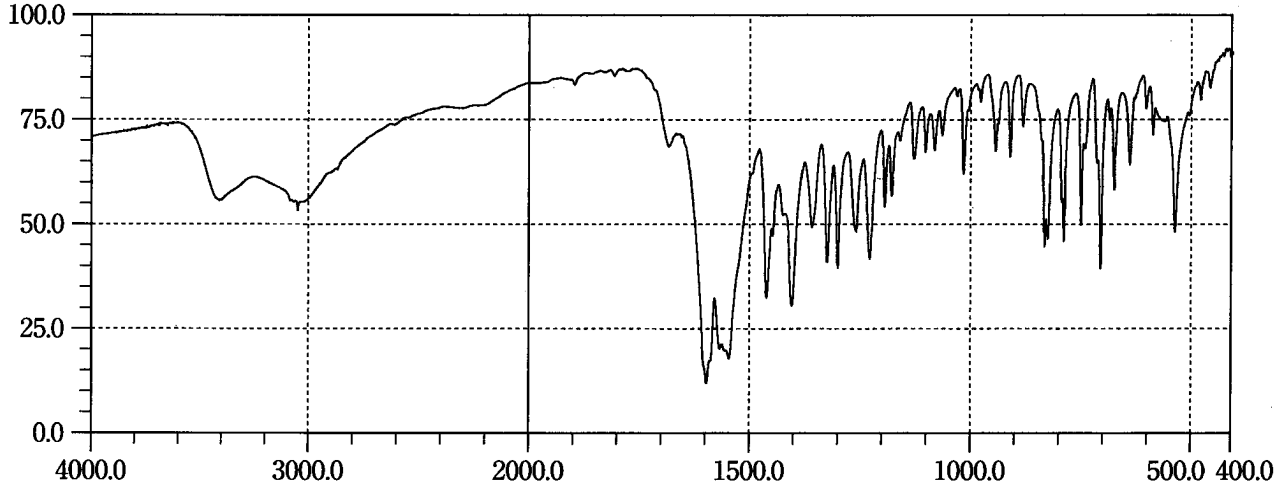
Cilazapril Hydrate



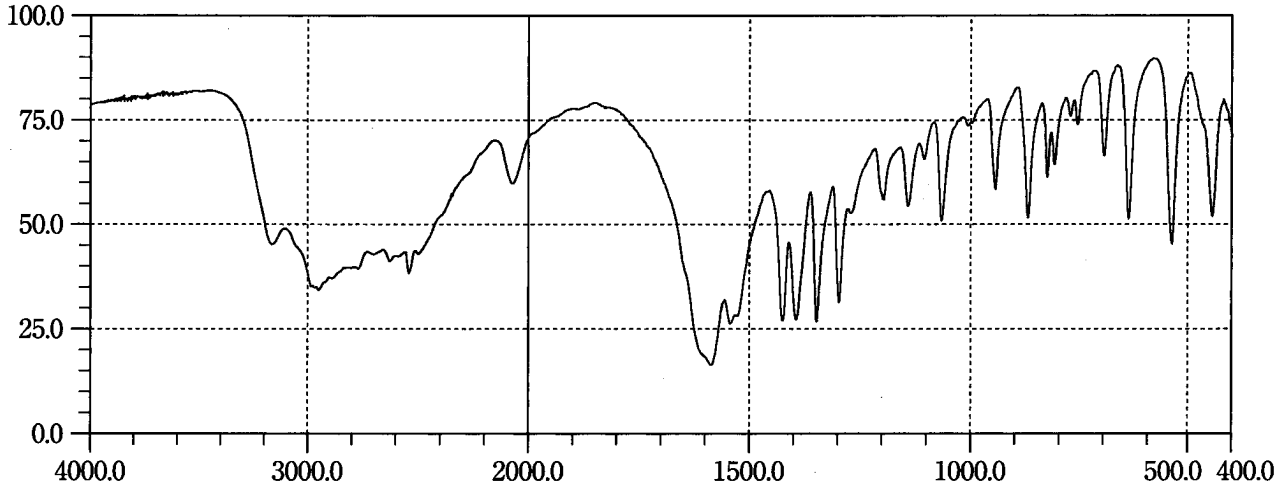
Clobetasol Propionate



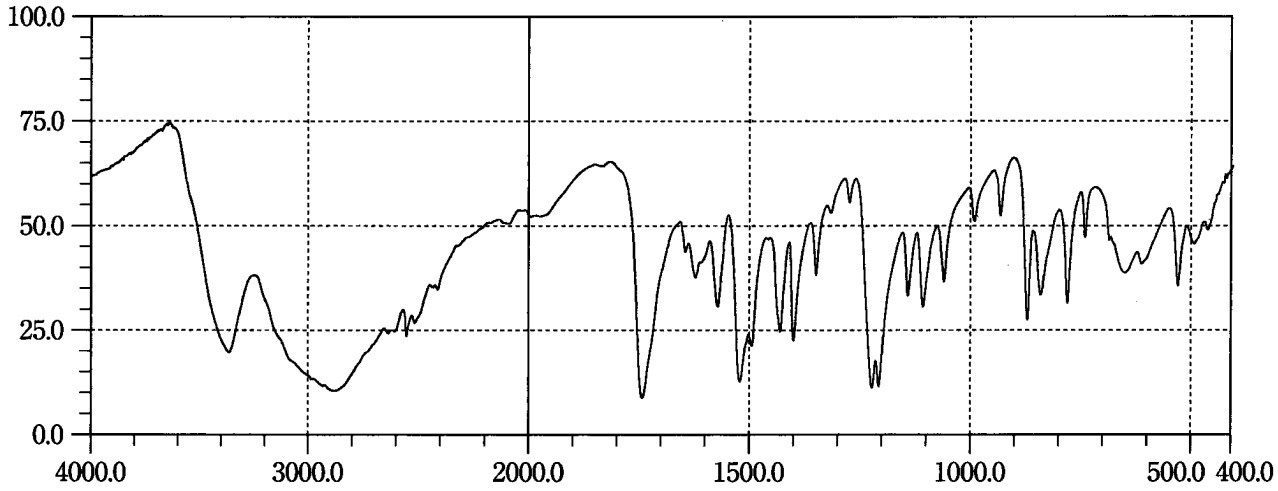
Clorazepate Dipotassium



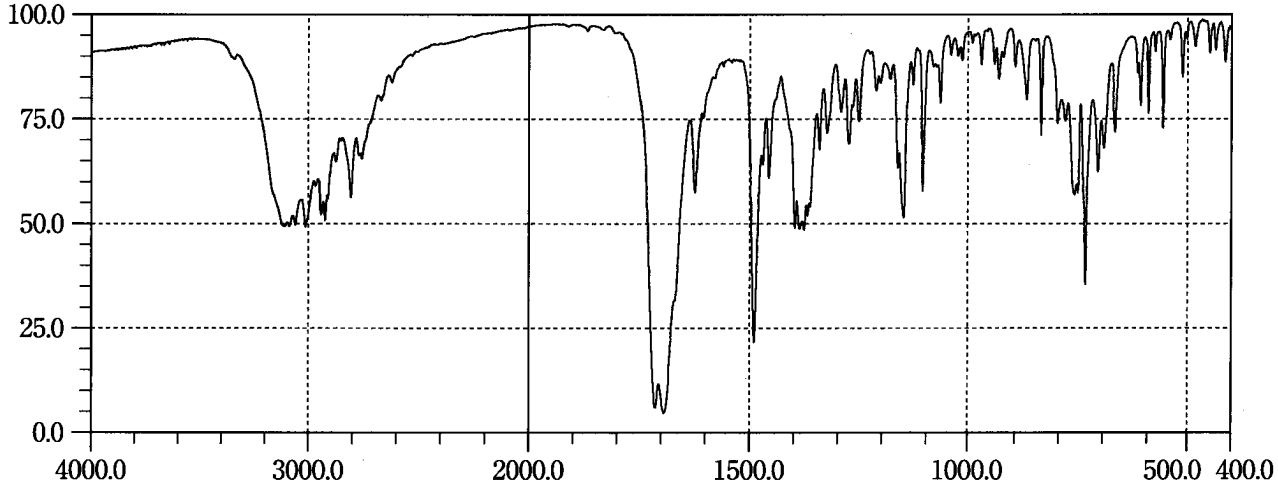
L-Cysteine



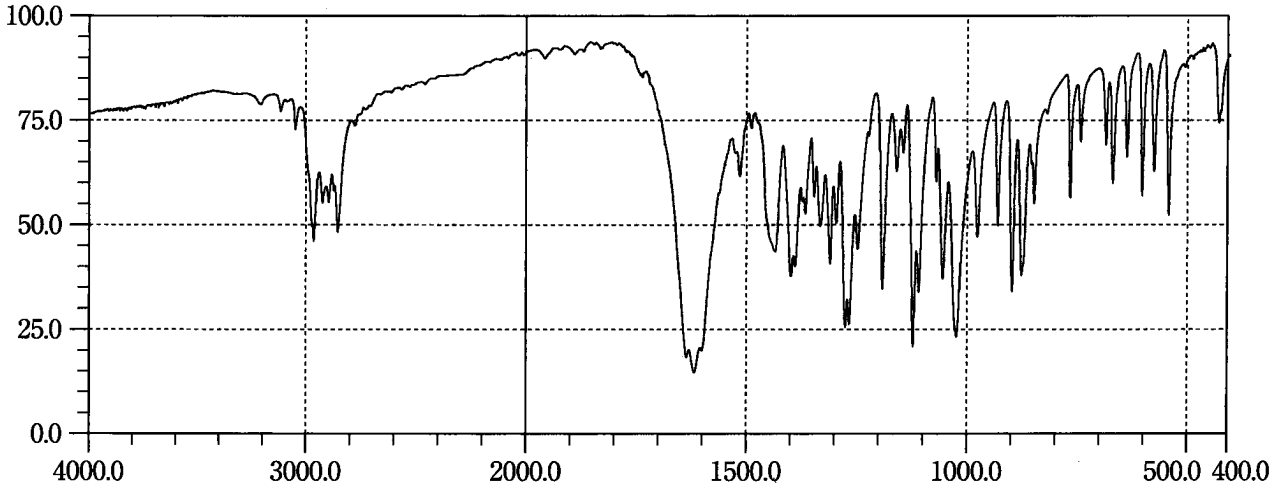
L-Cysteine Hydrochloride Hydrate



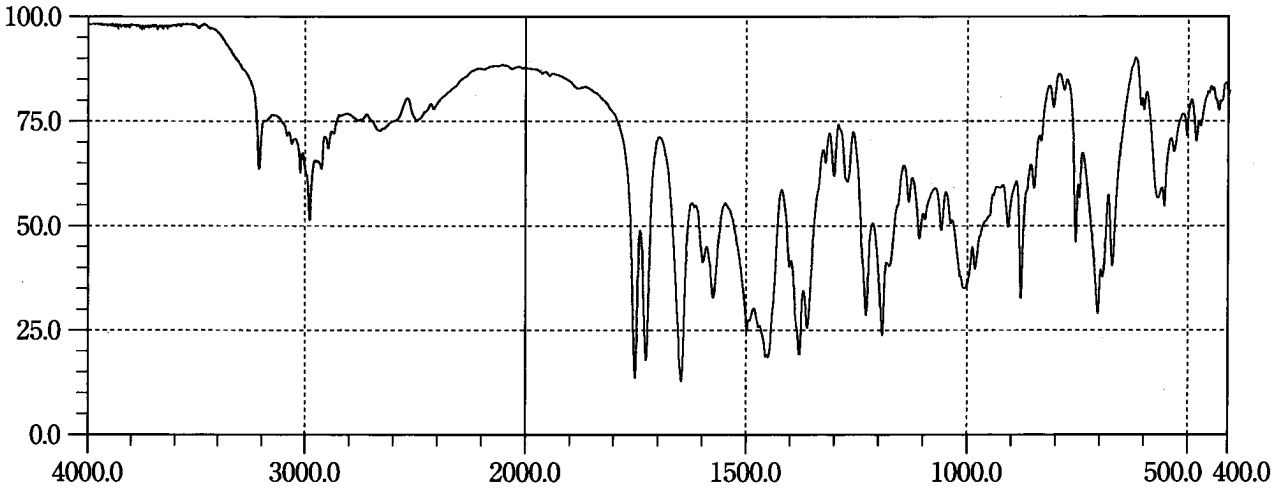
Domperidone



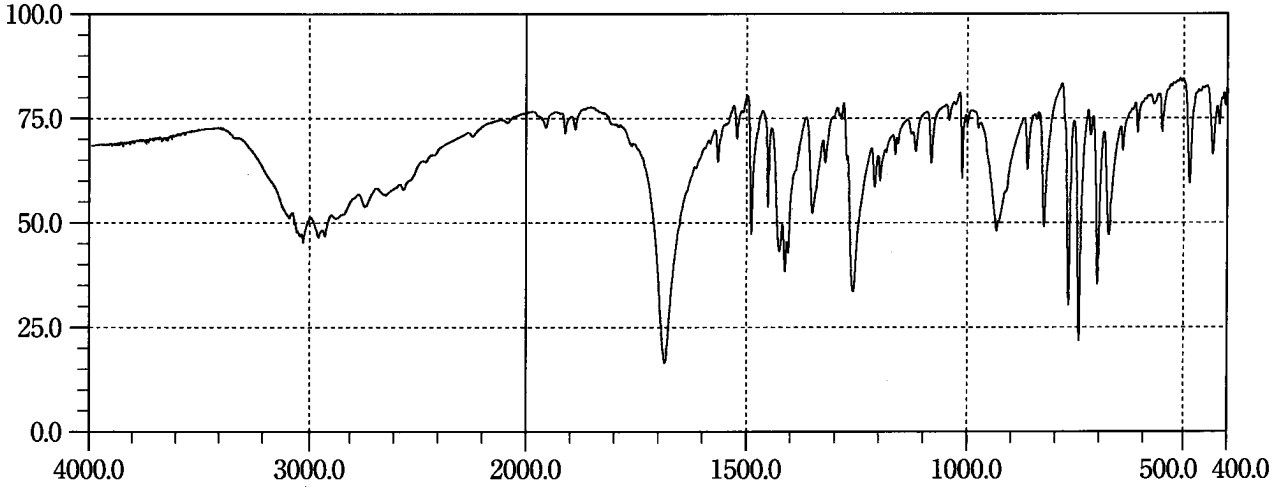
Emorfazone



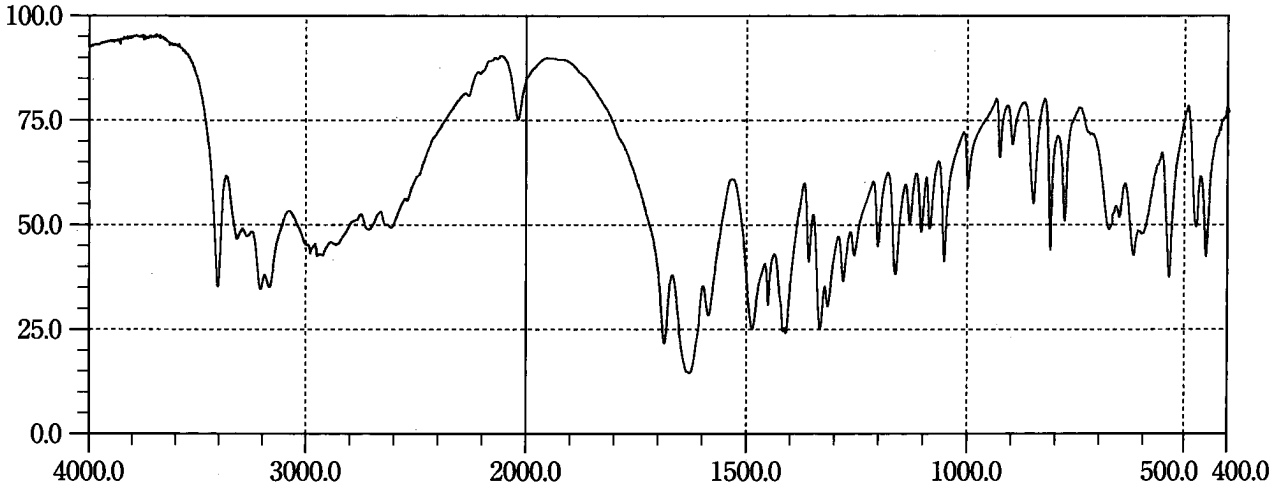
Enalapril Maleate



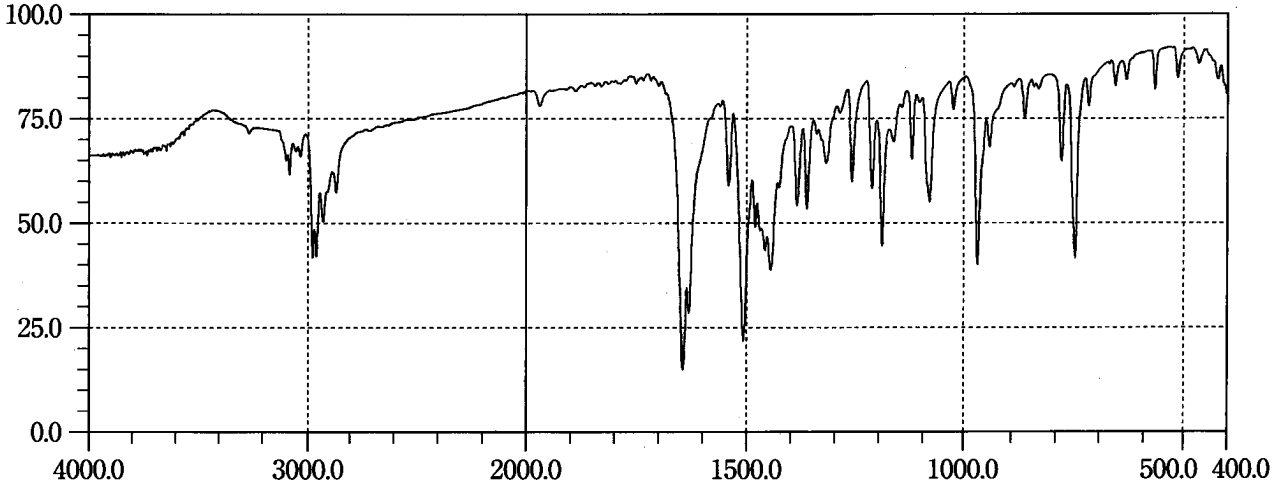
Felbinac



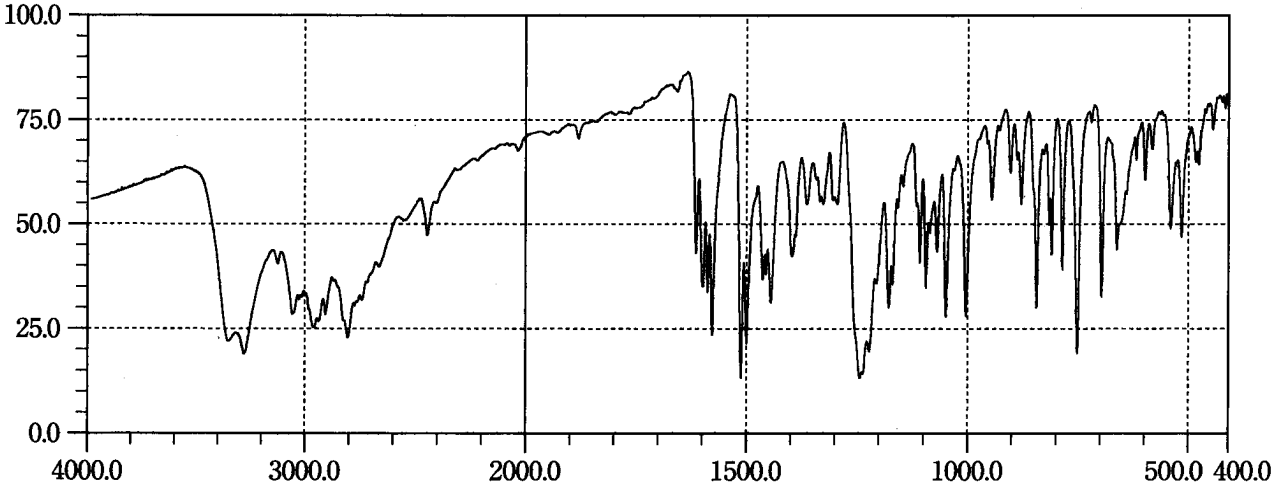
L-Glutamine



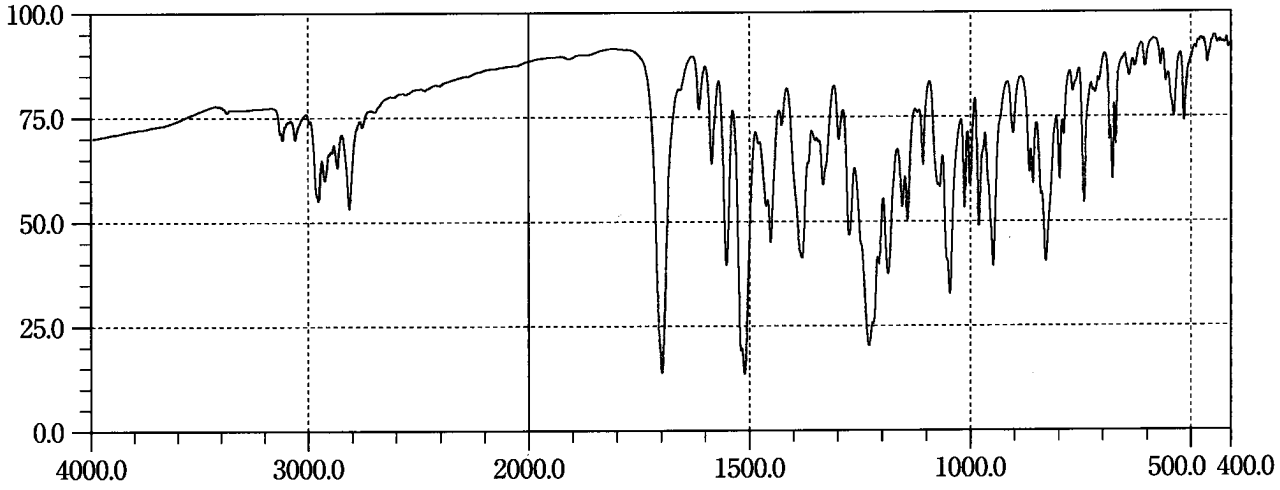
Ibudilast



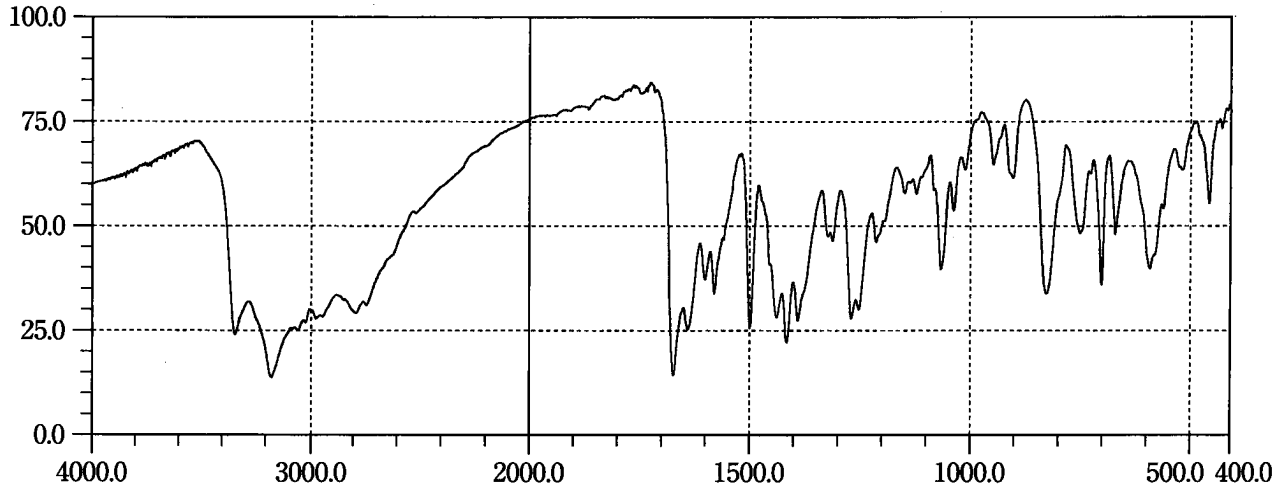
Isoxsuprine Hydrochloride



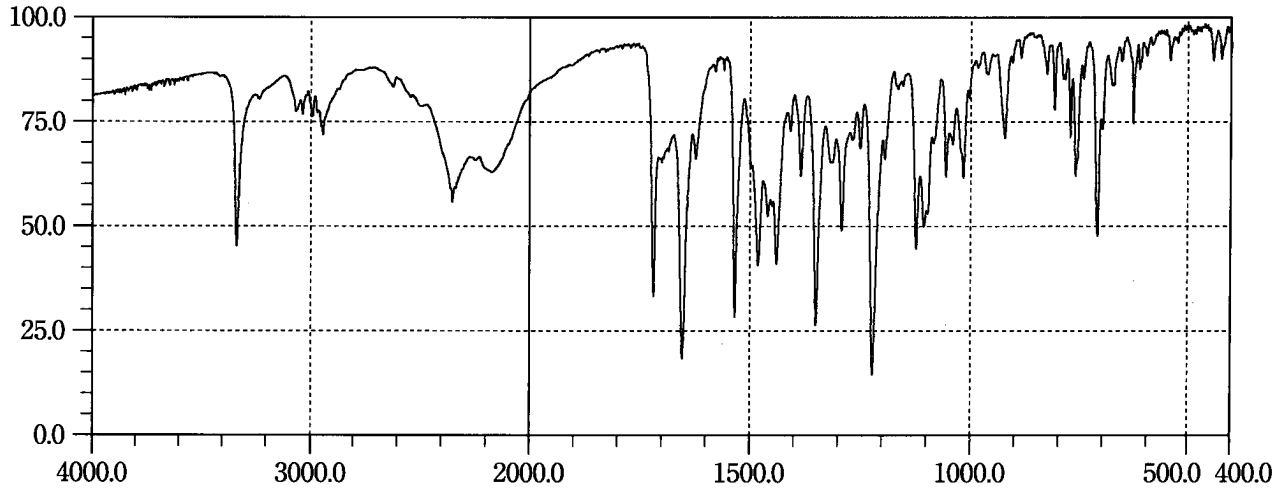
Itraconazole



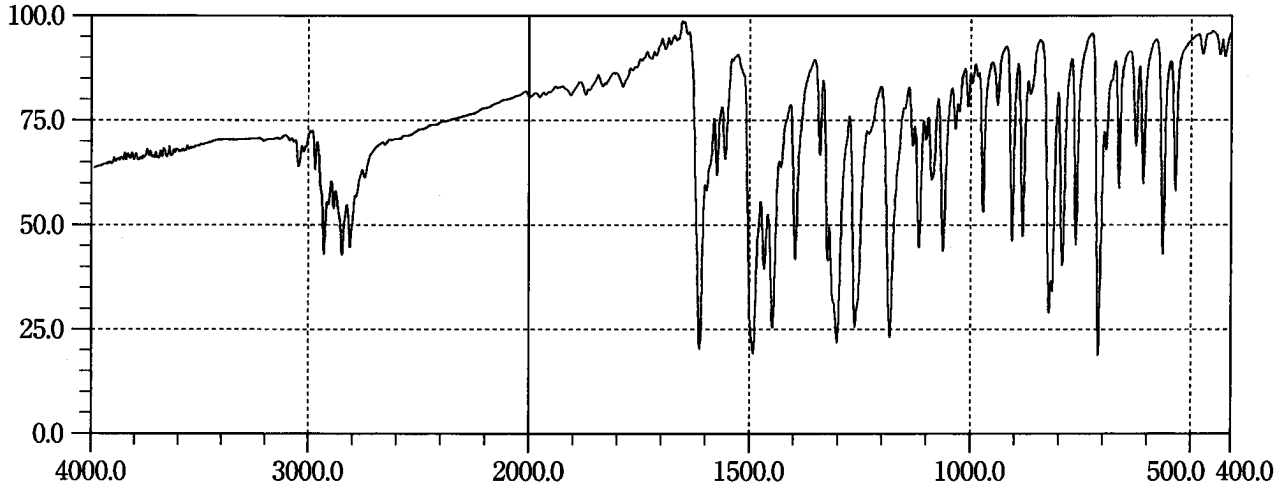
Labetalol Hydrochloride



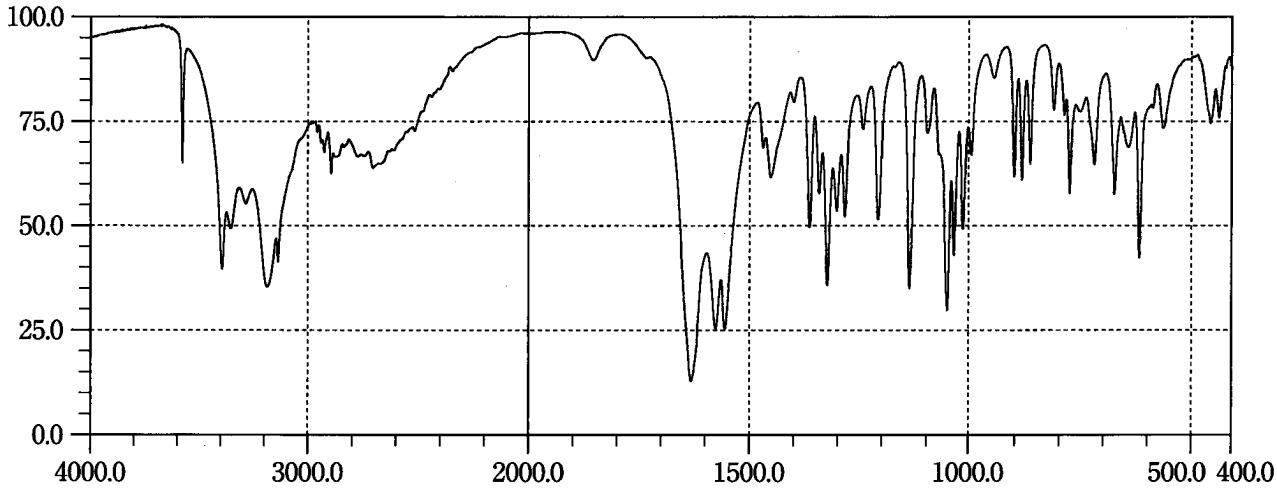
Manidipine Hydrochloride



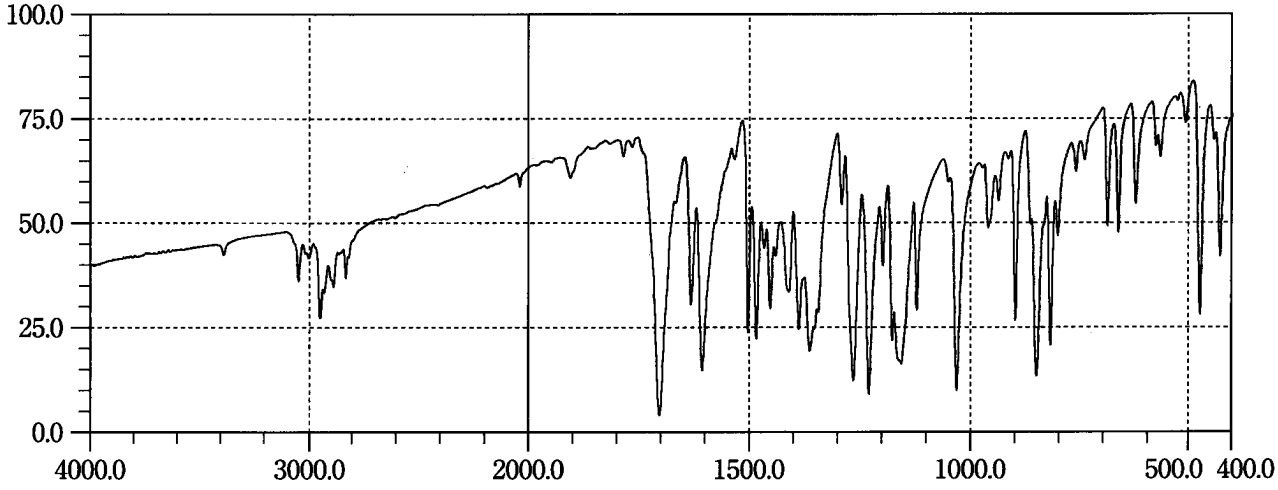
Medazepam



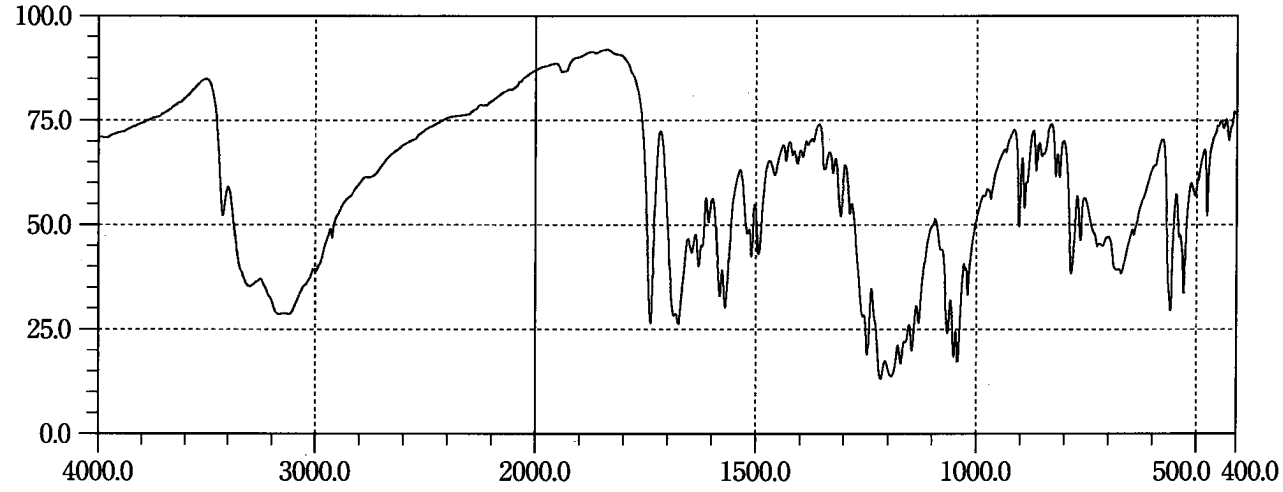
Mizoribine



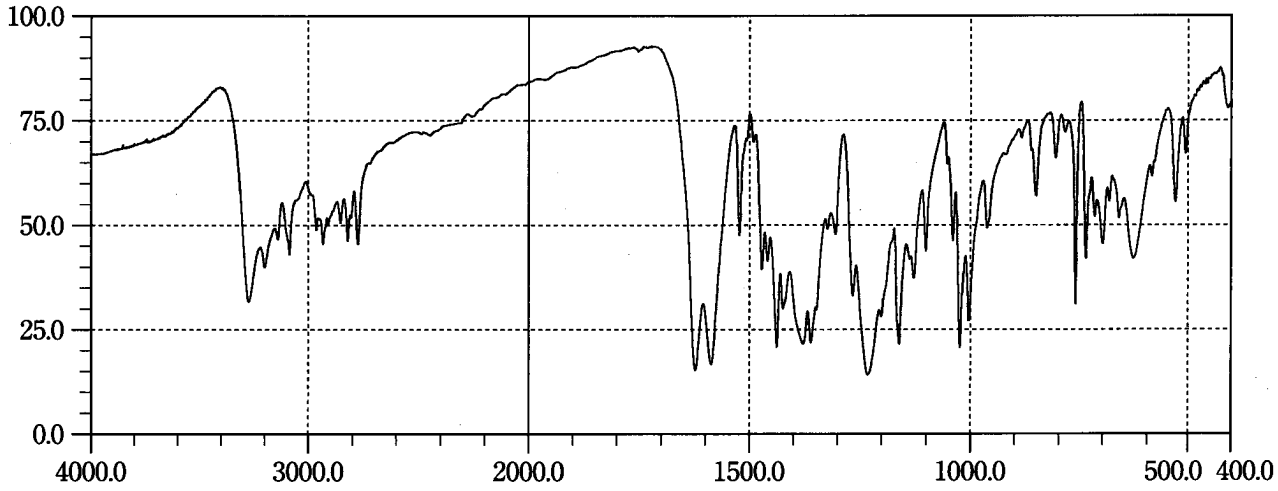
Nabumetone



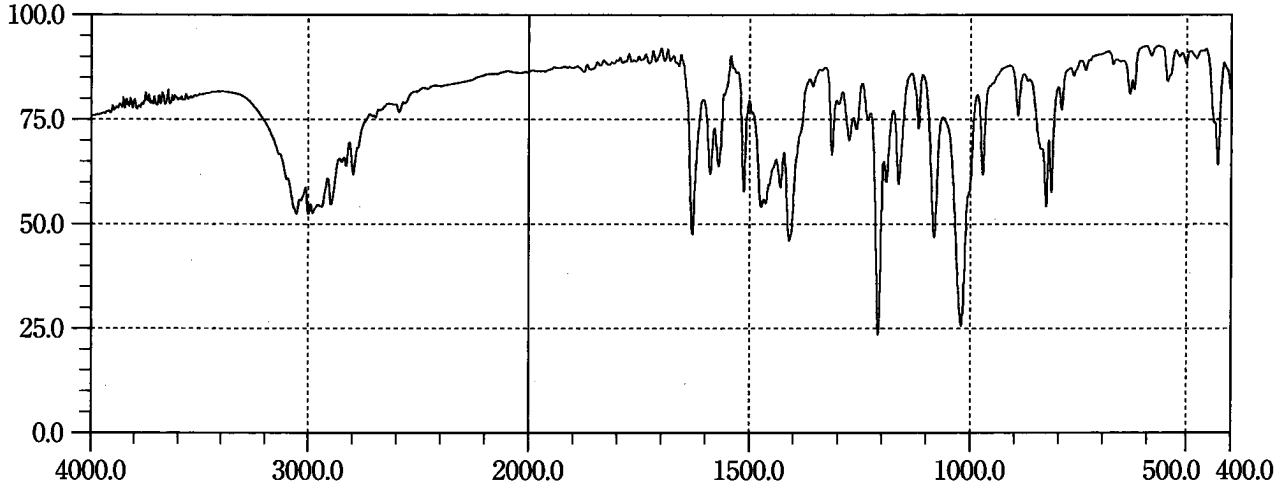
Nafamostat Mesilate



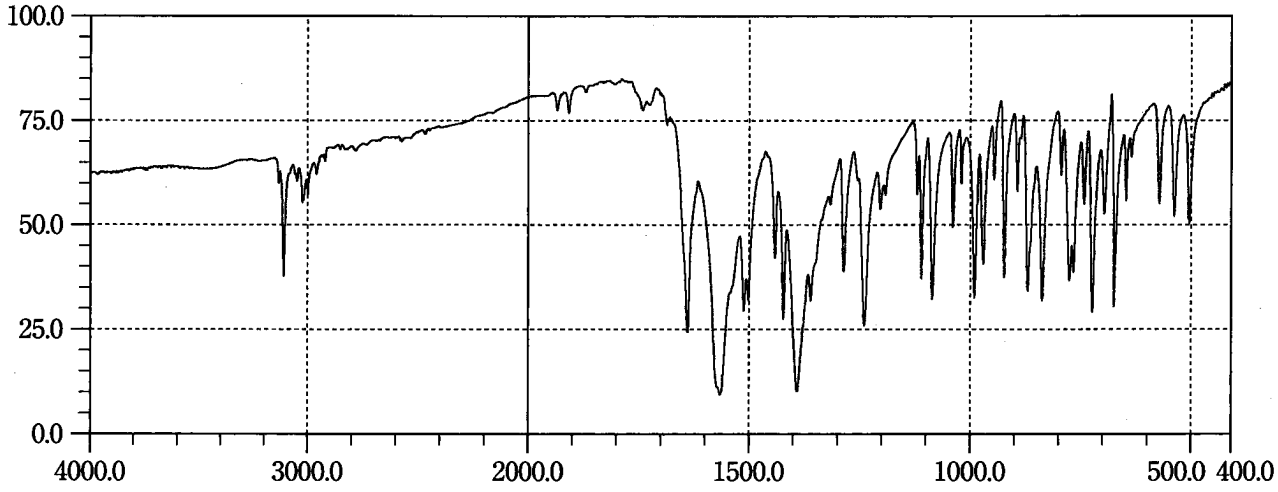
Nizatidine



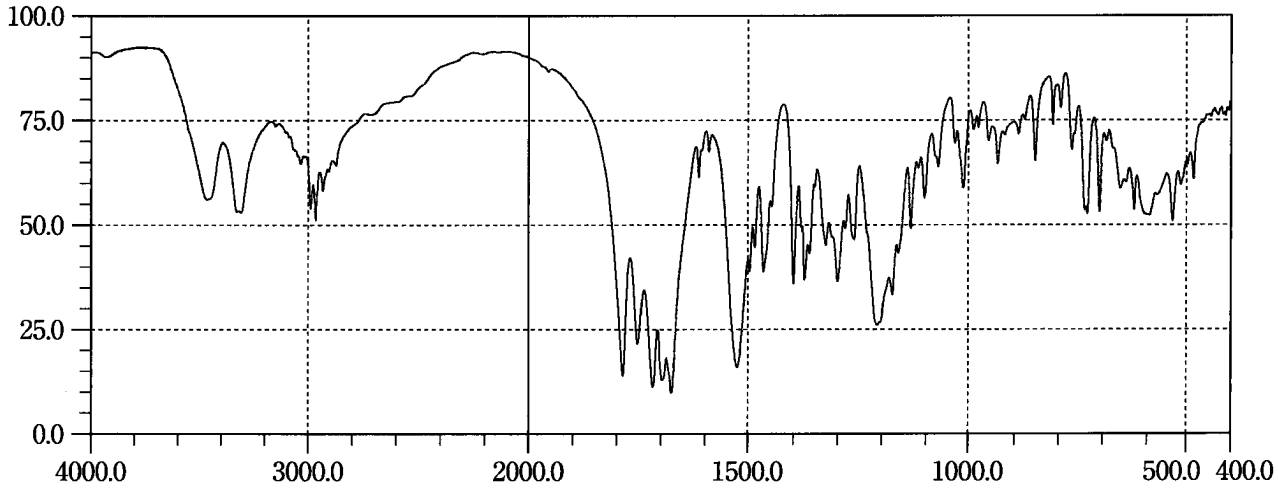
Omeprazole



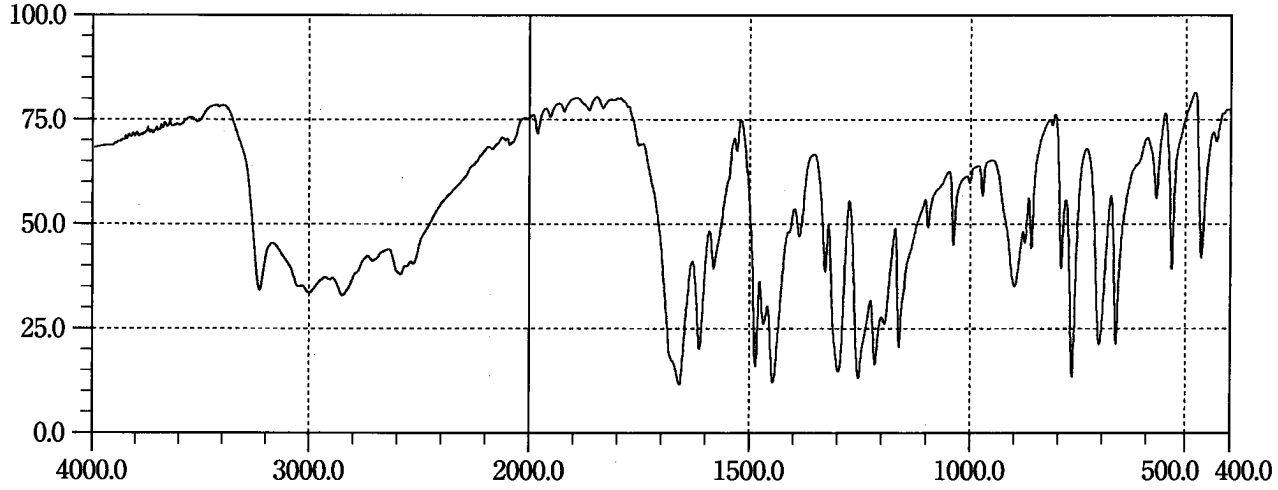
Ozagrel Sodium



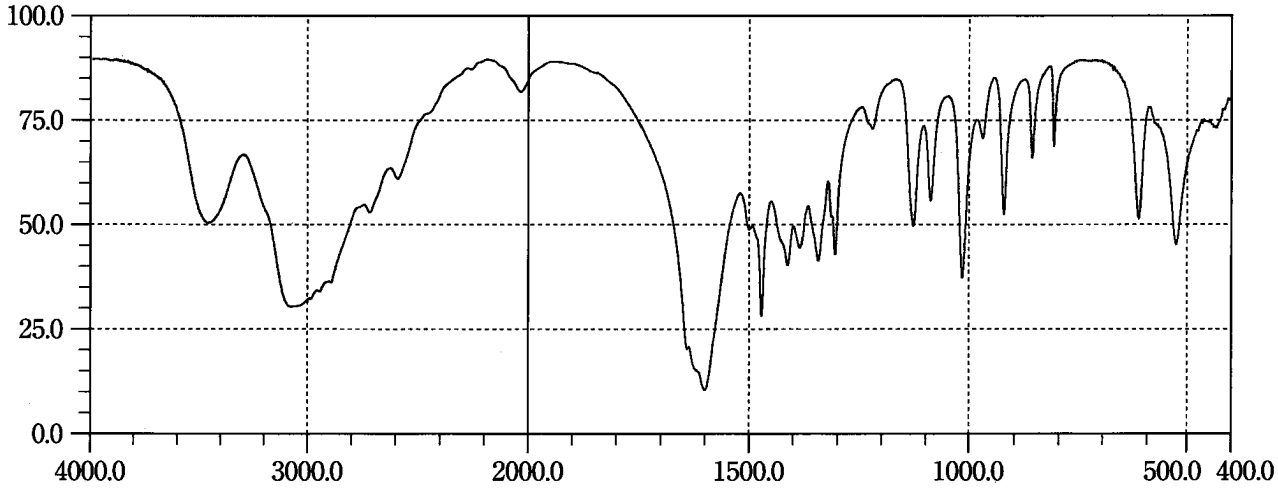
Piperacillin Hydrate



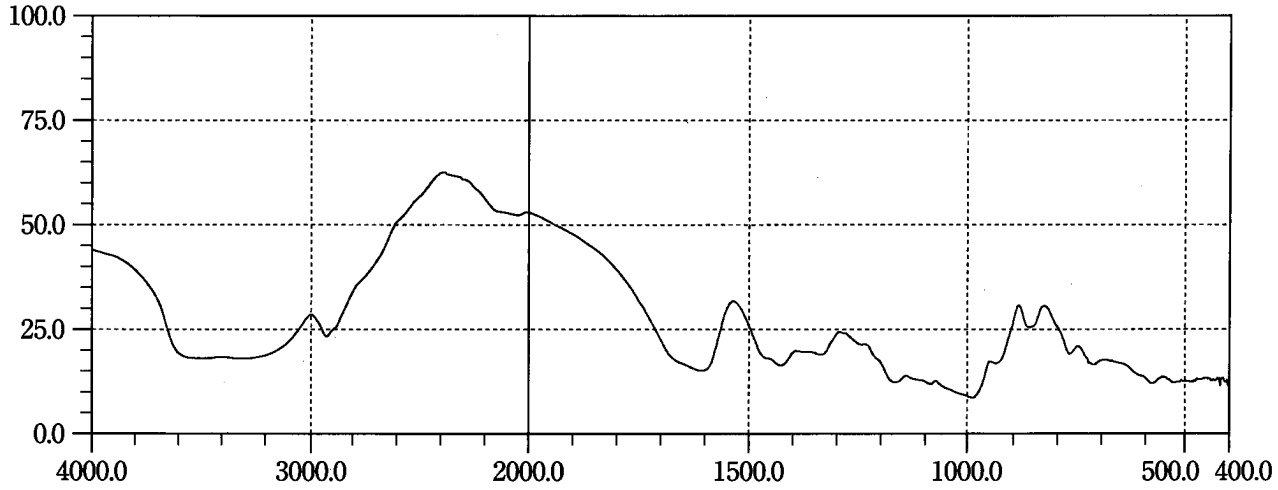
Salicylic Acid



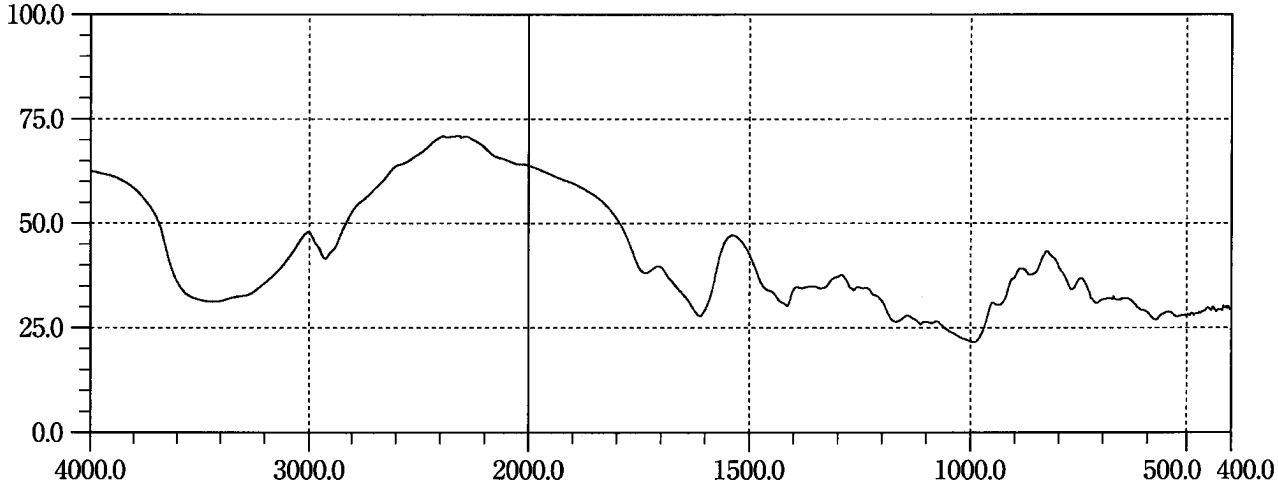
L-Serine



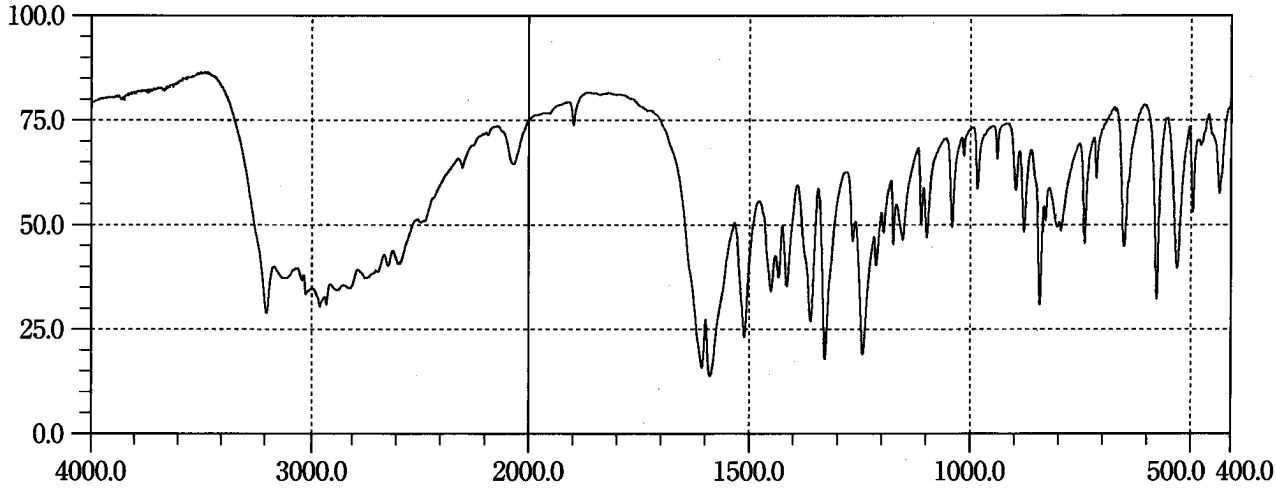
Sodium Starch Glycolate, Type A



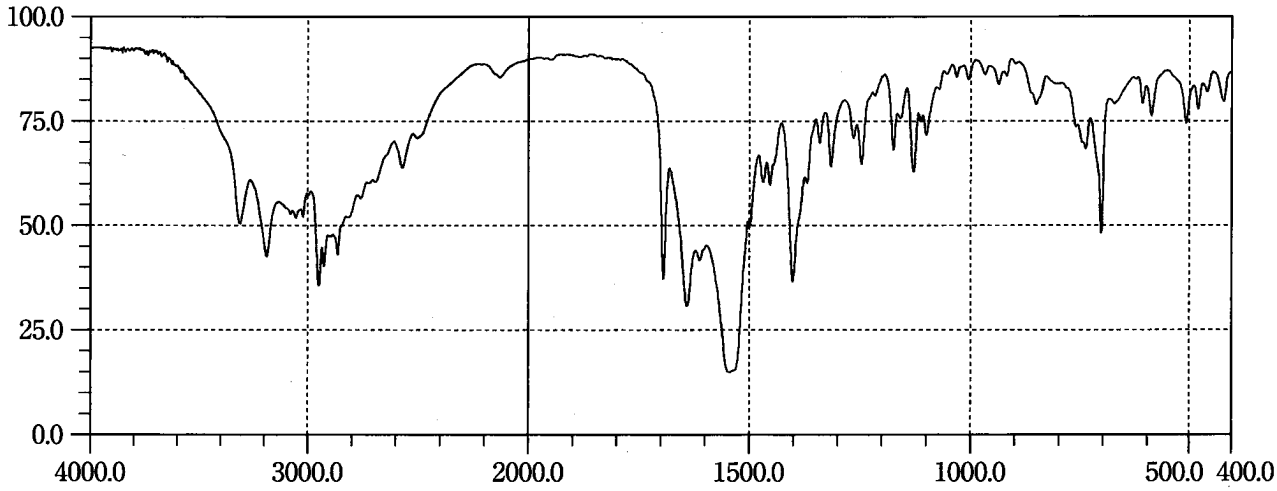
Sodium Starch Glycolate, Type B



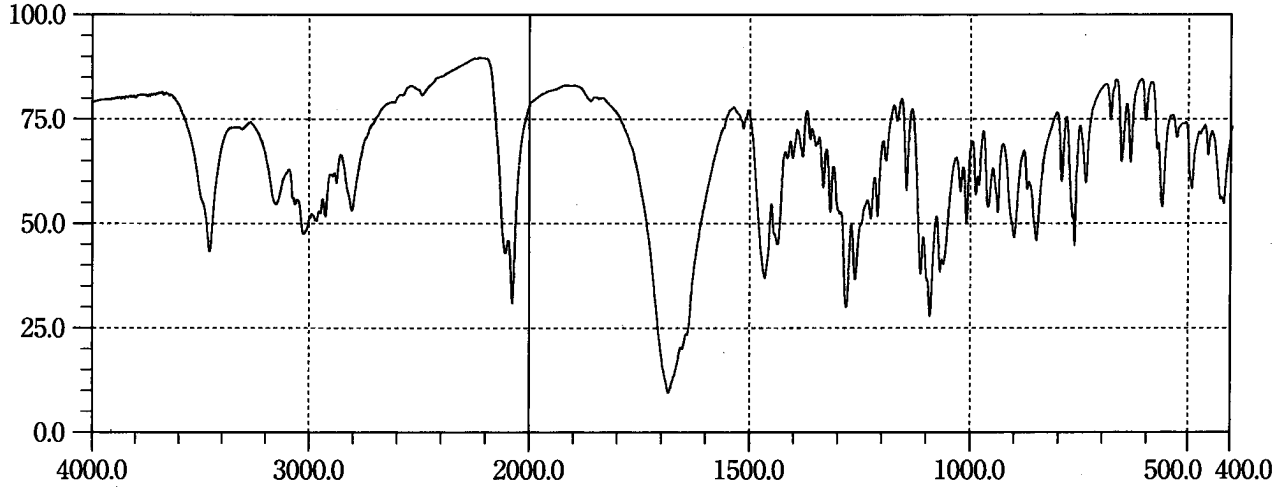
L-Tyrosine



Ubenimex



Zidovudine



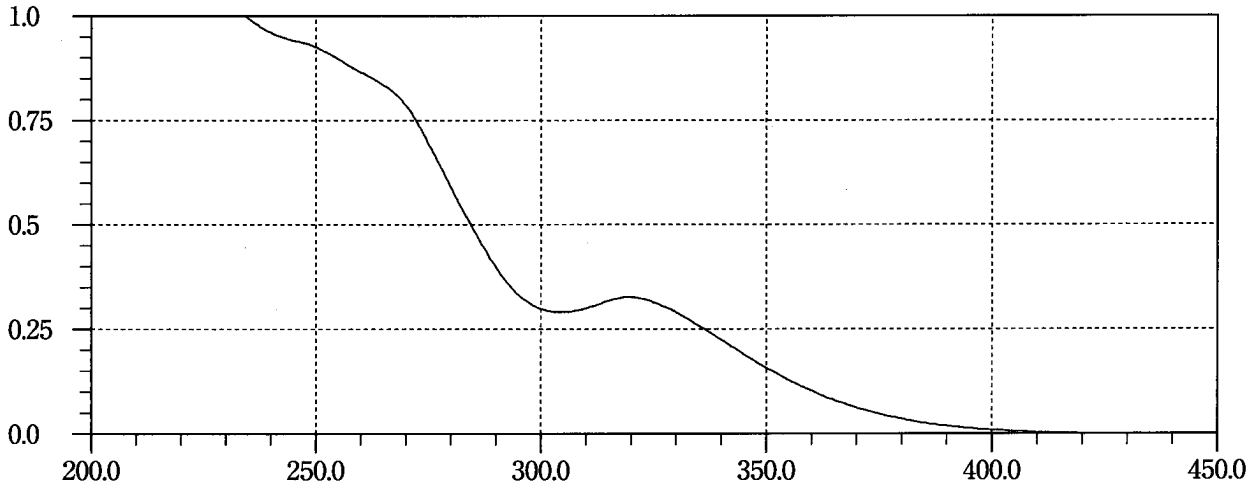
Delete the following Ultraviolet-visible Reference Spectra:

Sulfinpyrazone

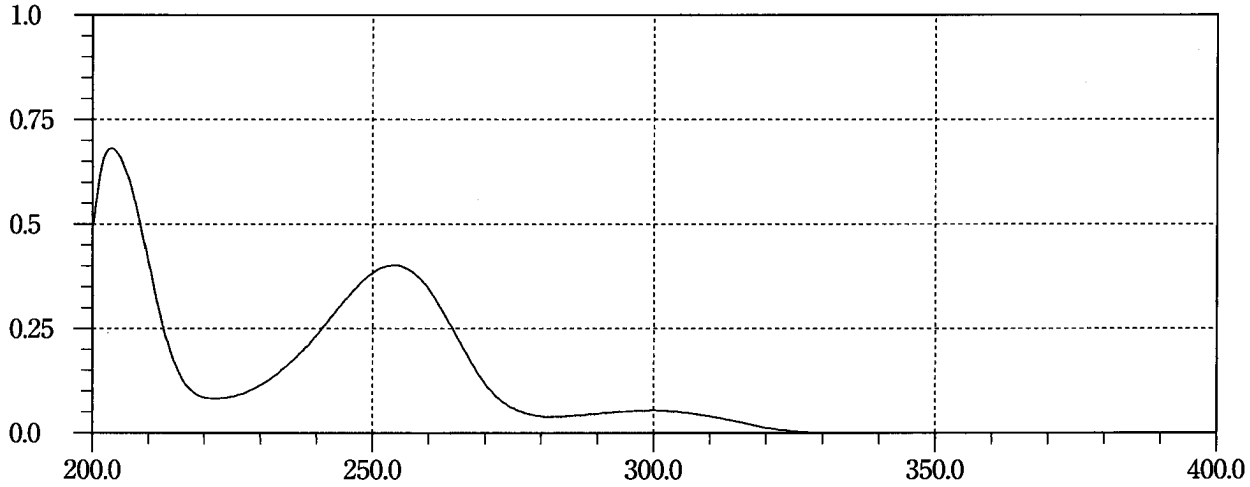
Tubocurarine Chloride Hydrochloride Hydrate

Add the following 30 spectra:

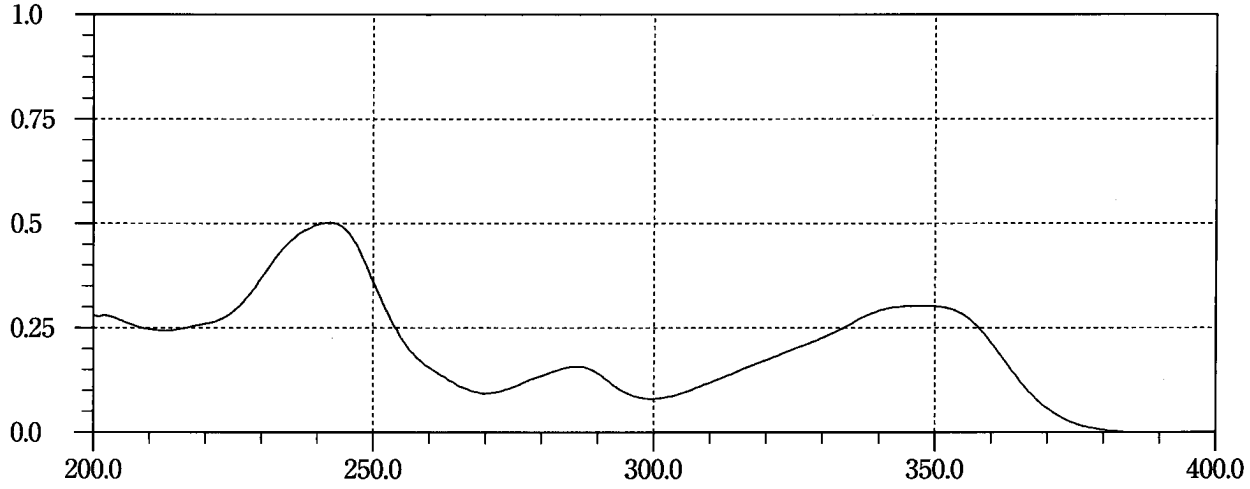
Acemetacin



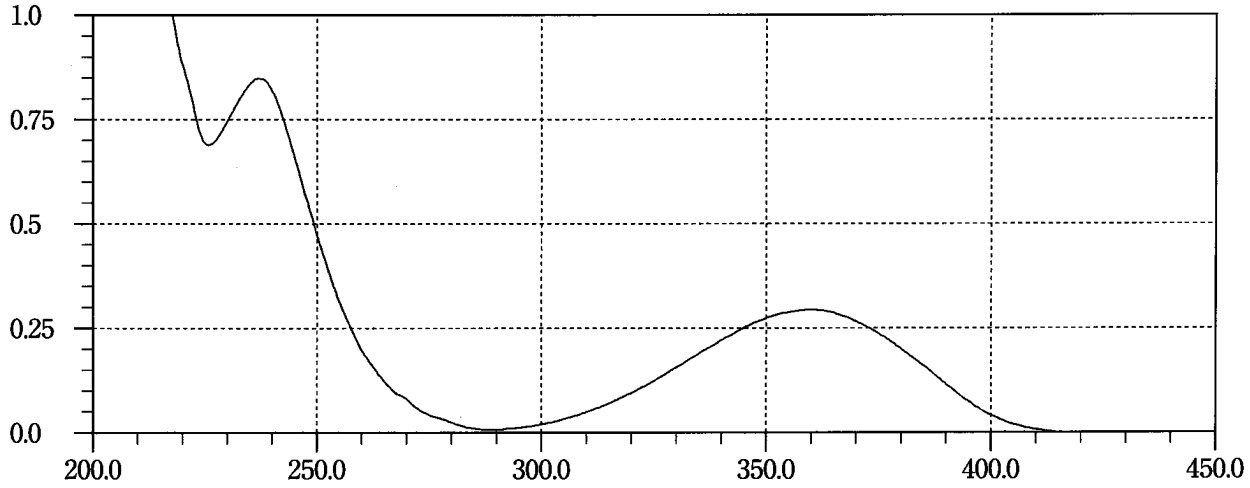
Alminoprofen



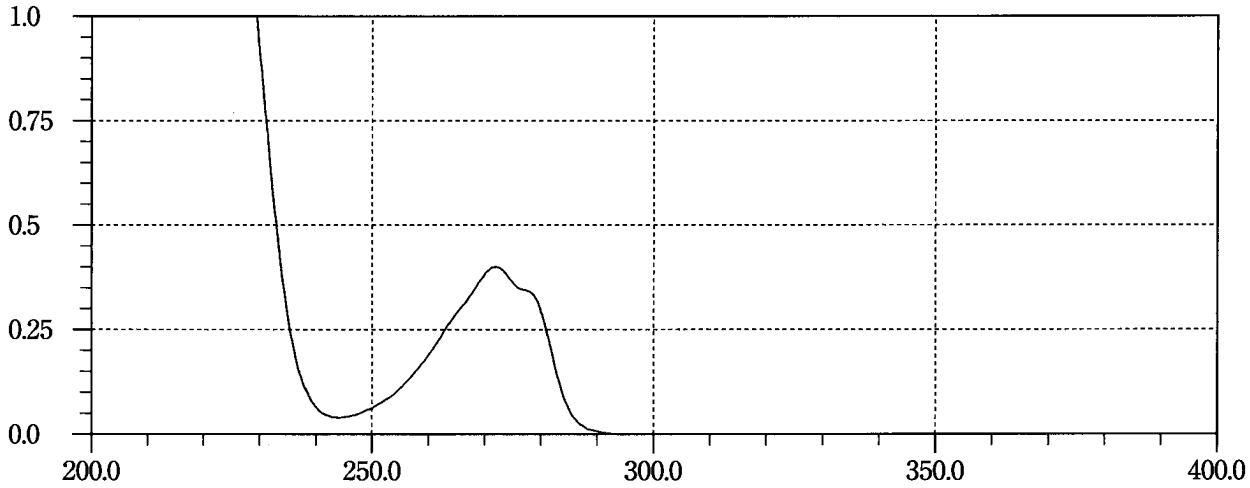
Amlexanox



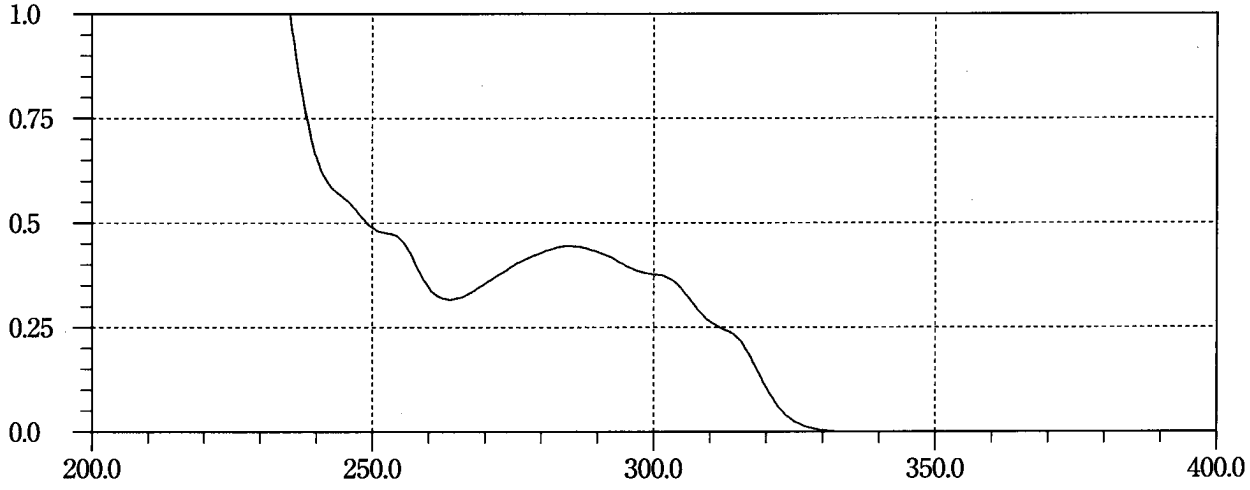
Amlodipine Besilate



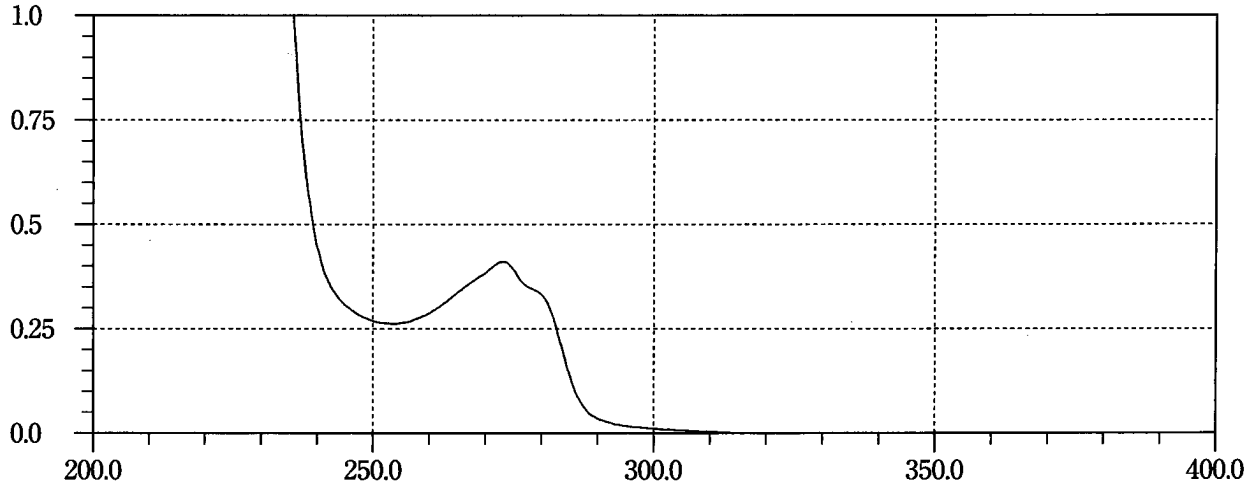
Amosulalol Hydrochloride



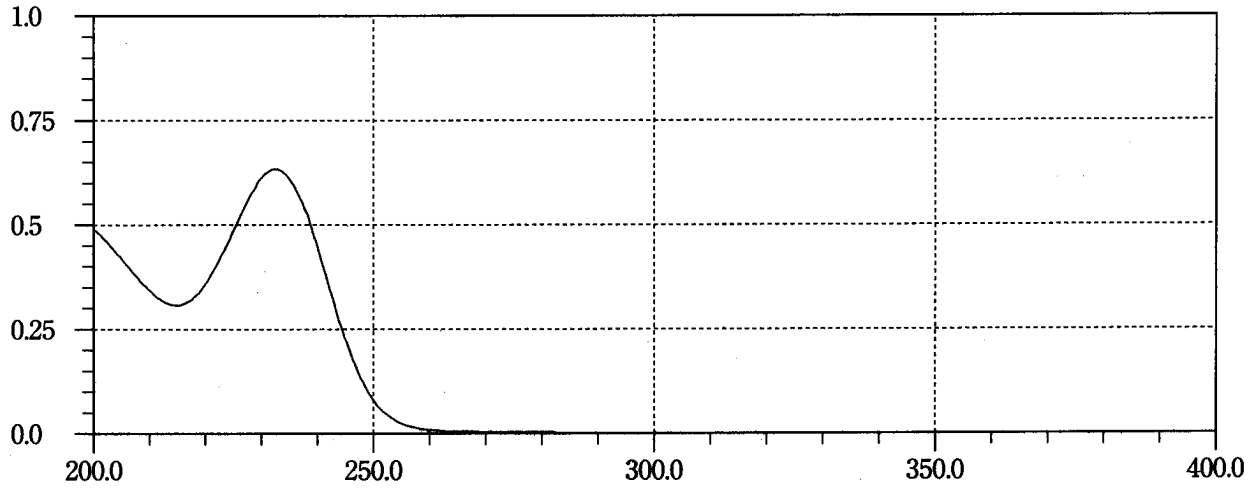
Azelastine Hydrochloride



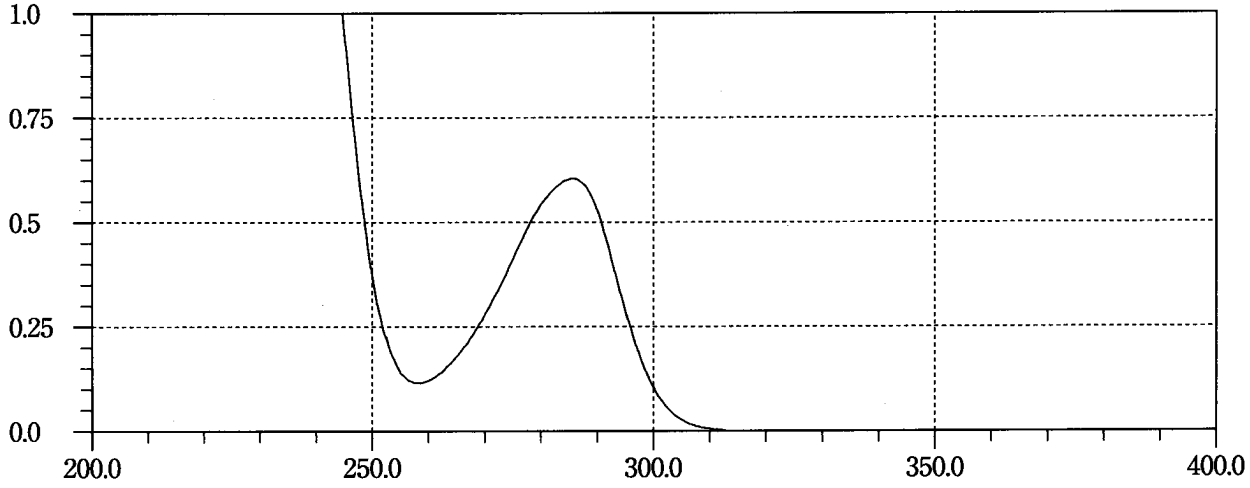
Bisoprolol Fumarate



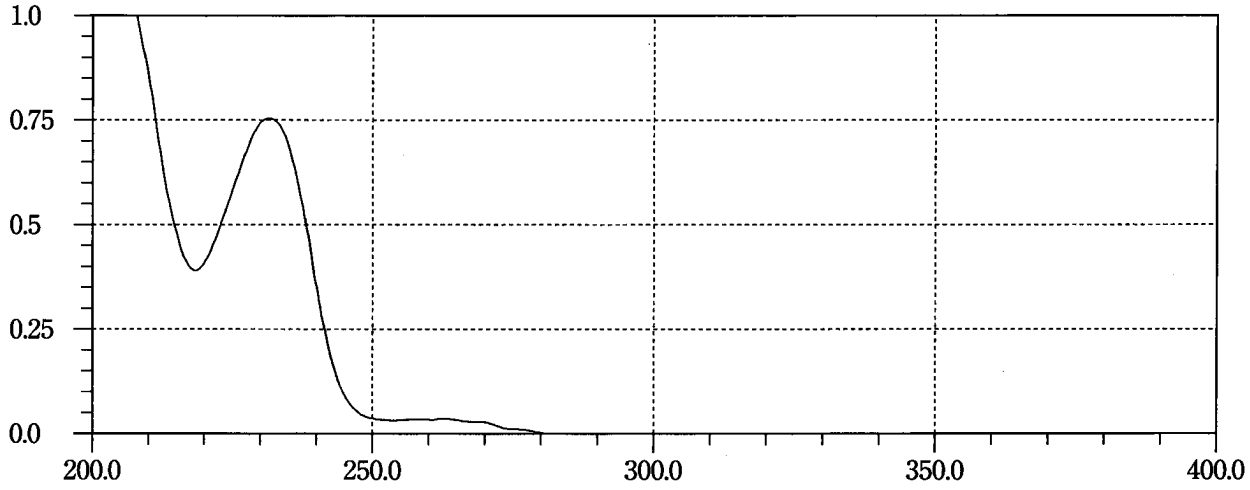
Buformin Hydrochloride



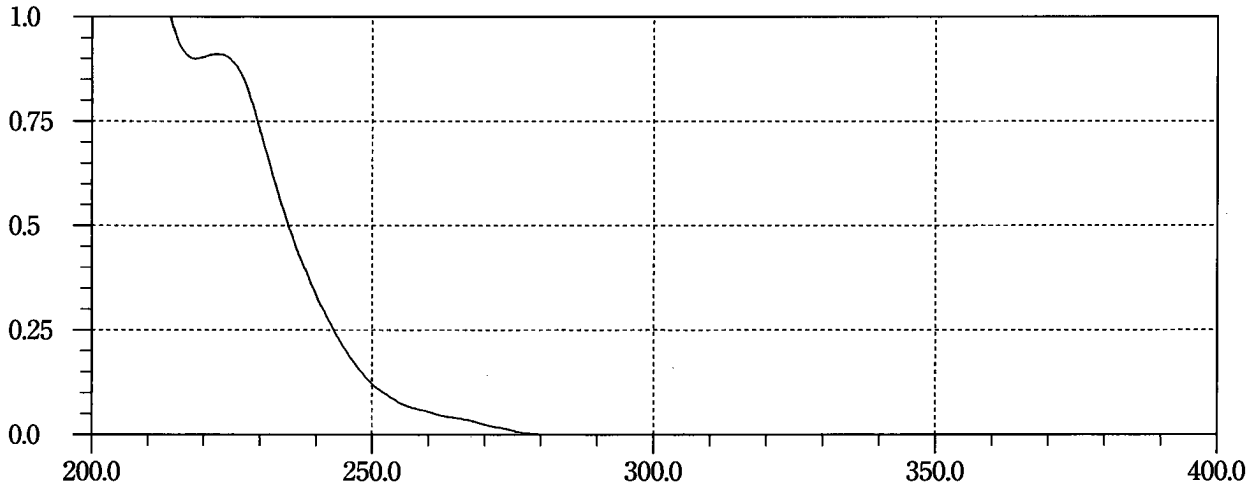
Buprenorphine Hydrochloride



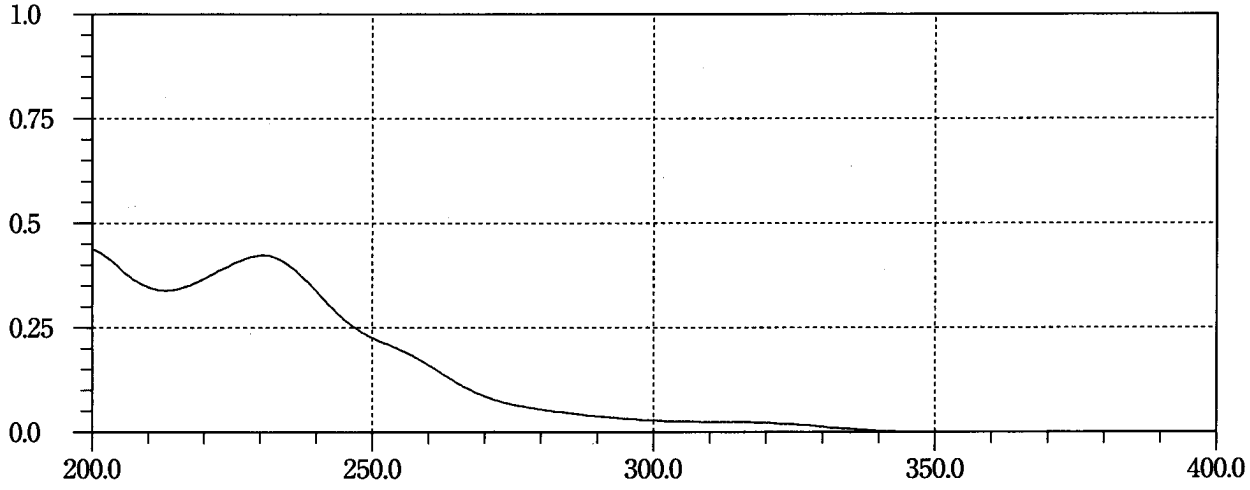
Cetirizine Hydrochloride



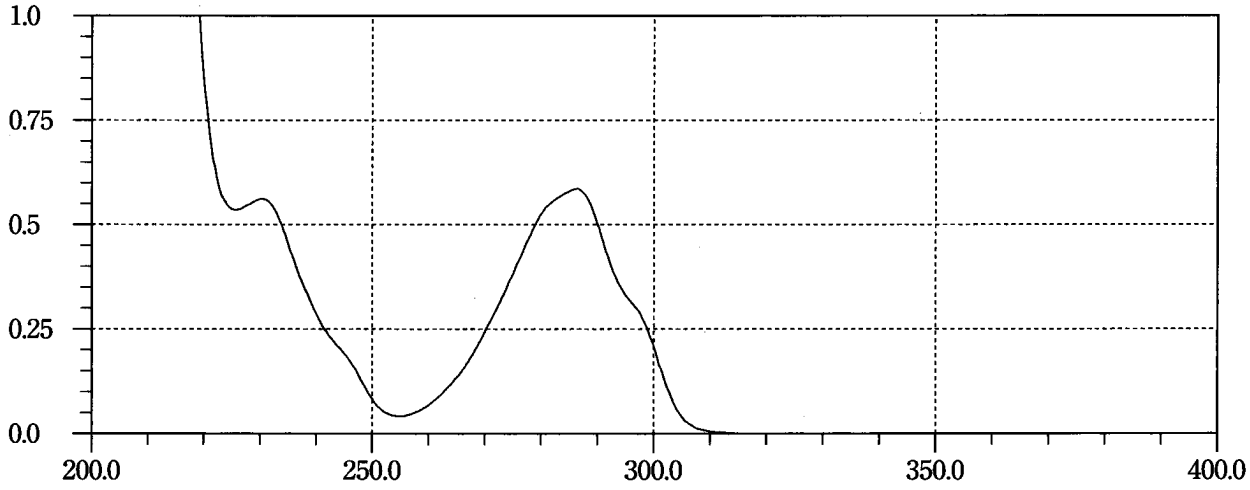
Cibenzoline Succinate



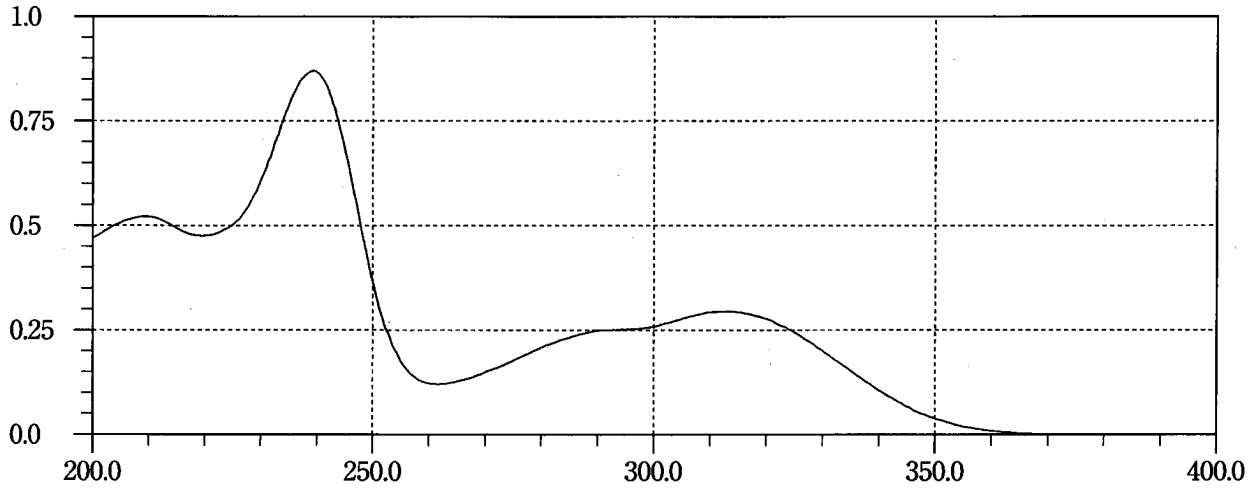
Clorazepate Dipotassium



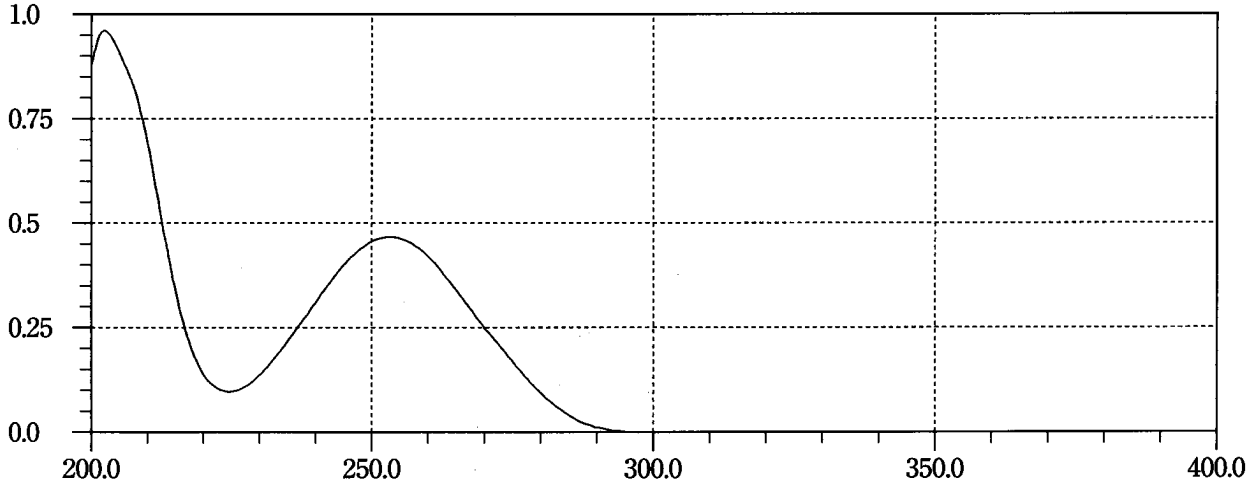
Domperidone



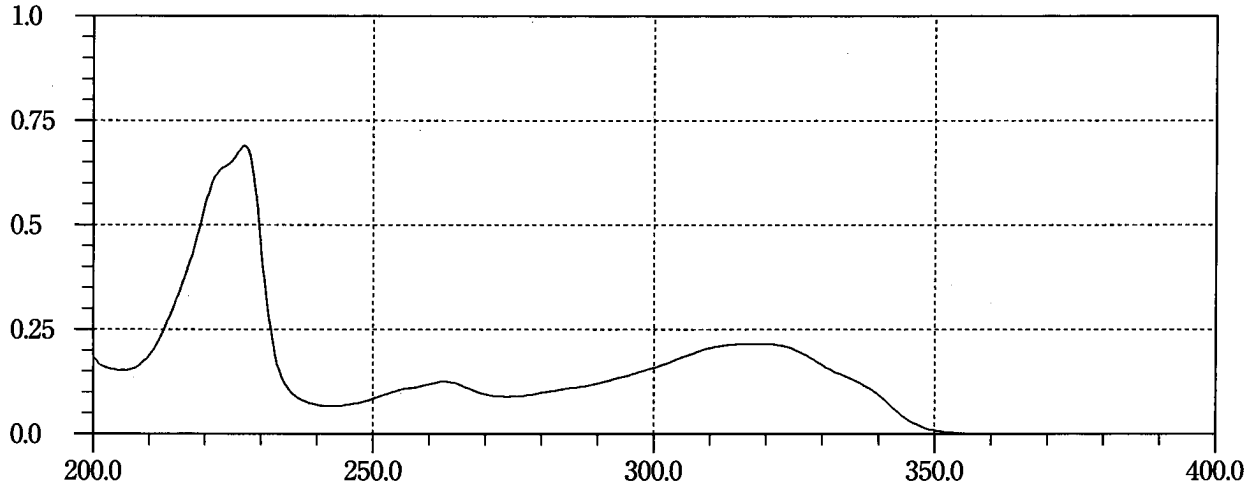
Emorfazone



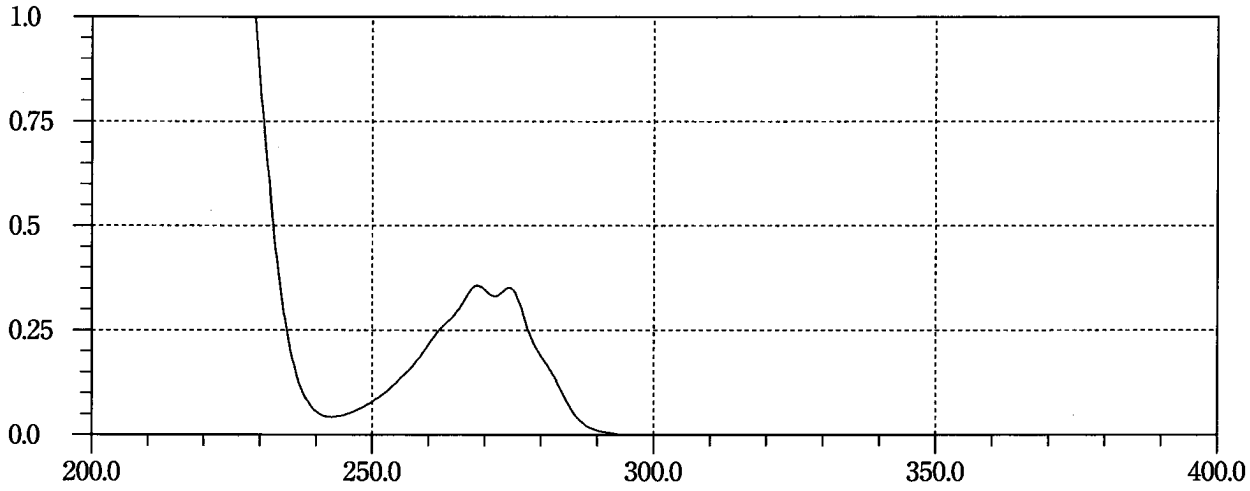
Felbinac



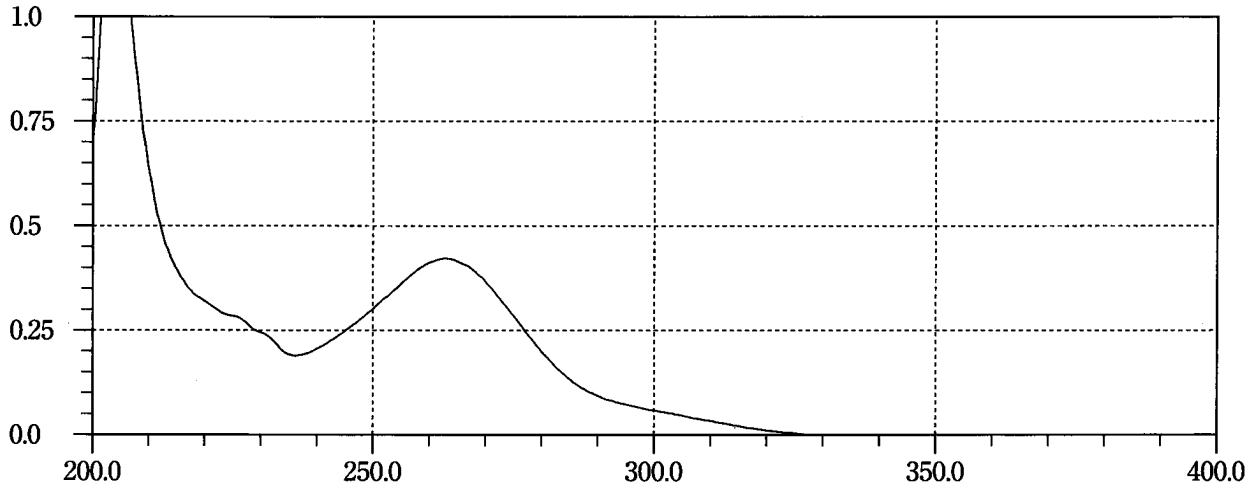
Ibudilast



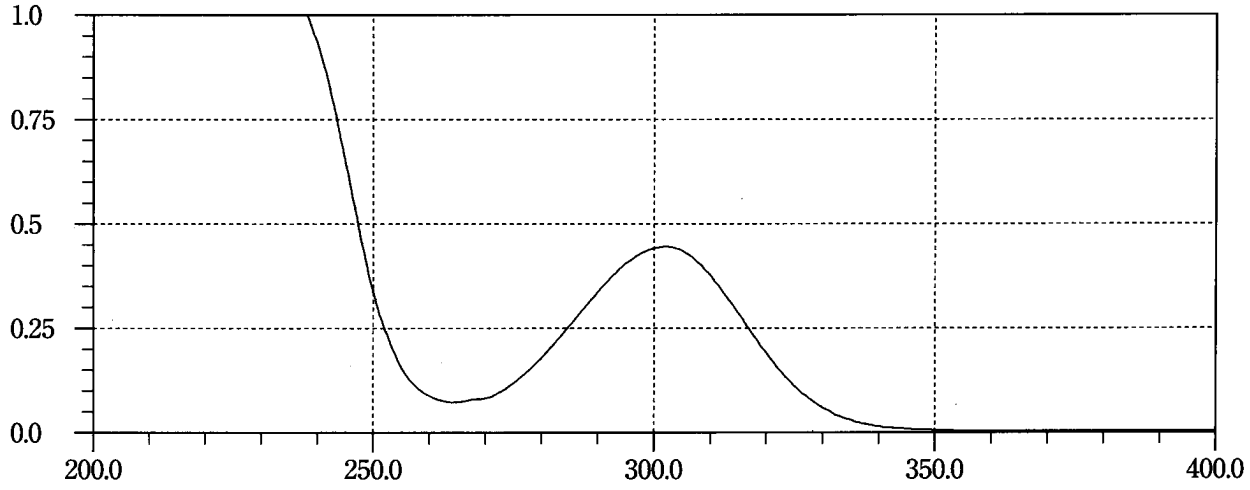
Isoxsuprine Hydrochloride



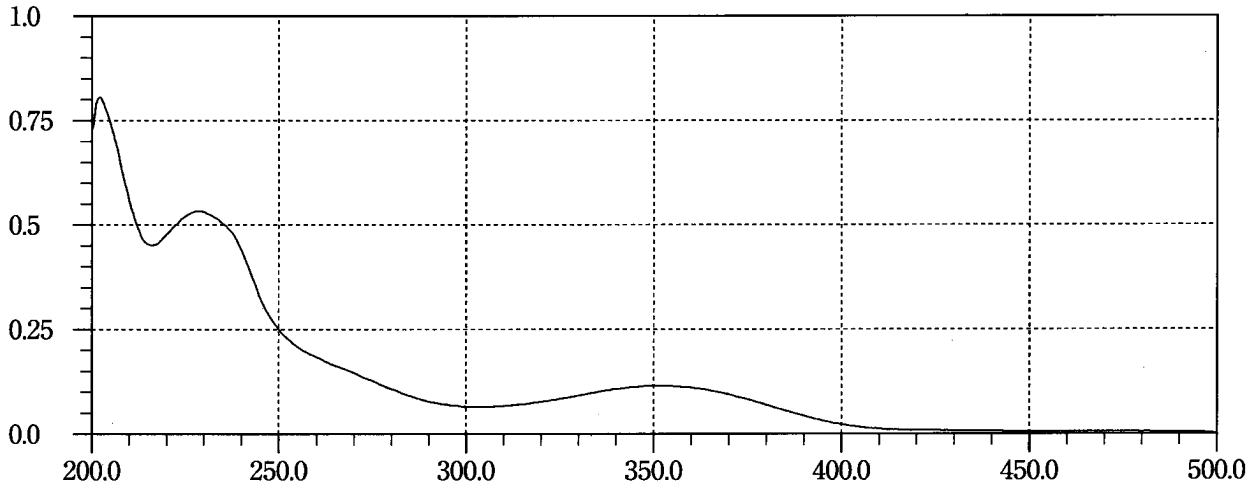
Itraconazole



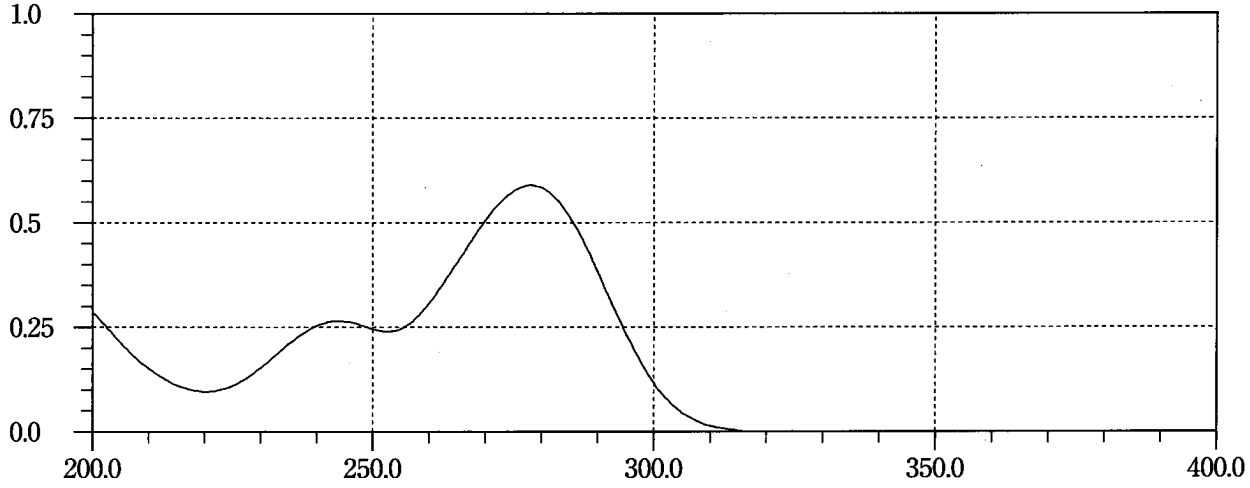
Labetalol Hydrochloride



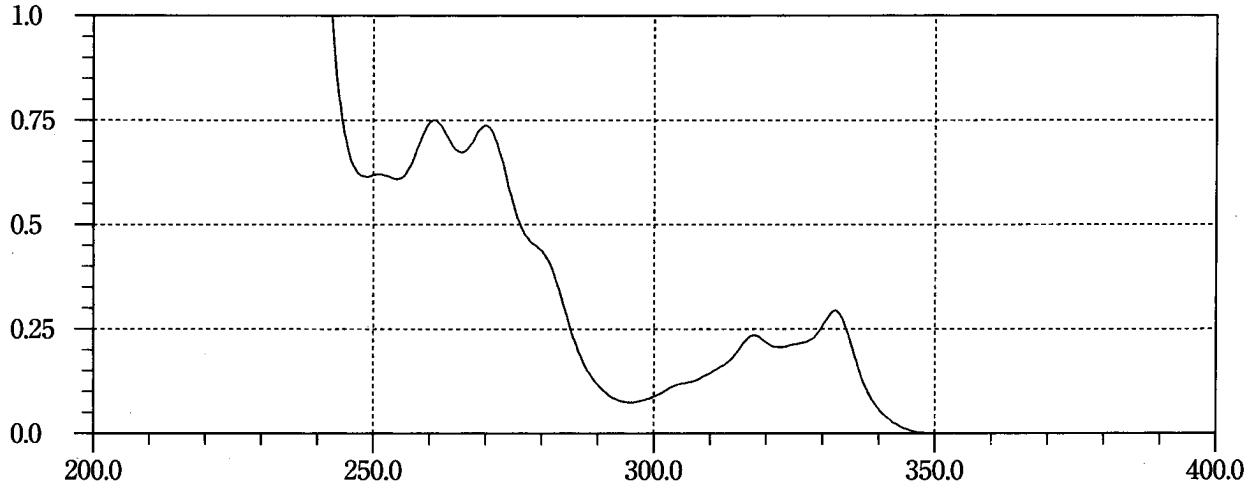
Manidipine Hydrochloride



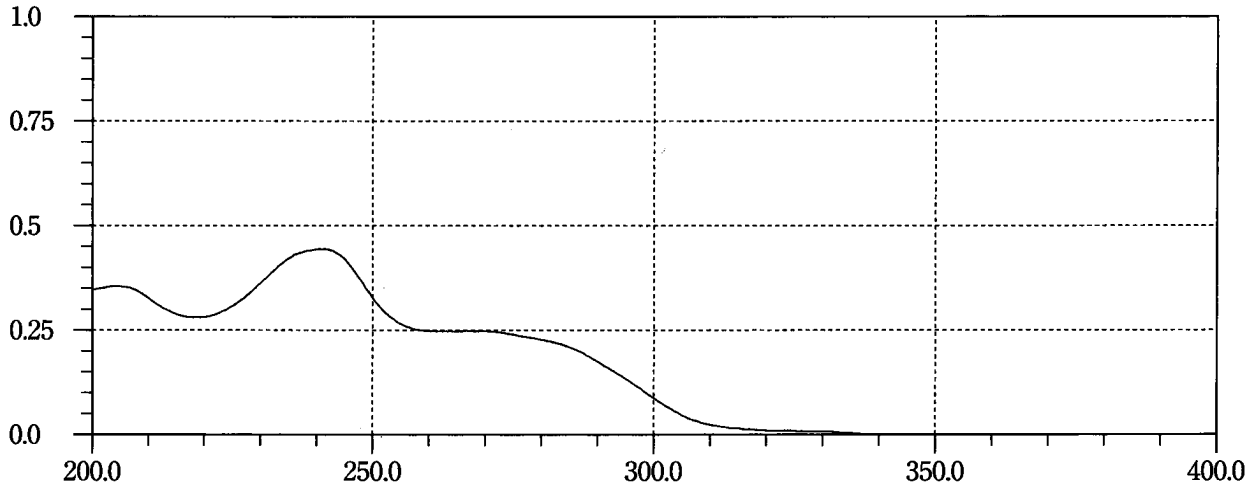
Mizoribine



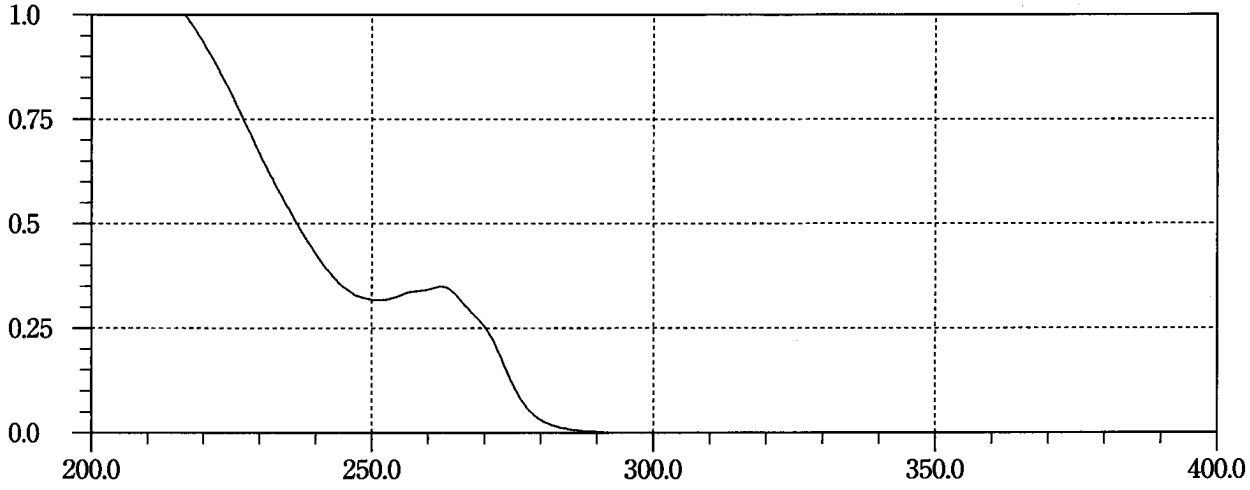
Nabumetone



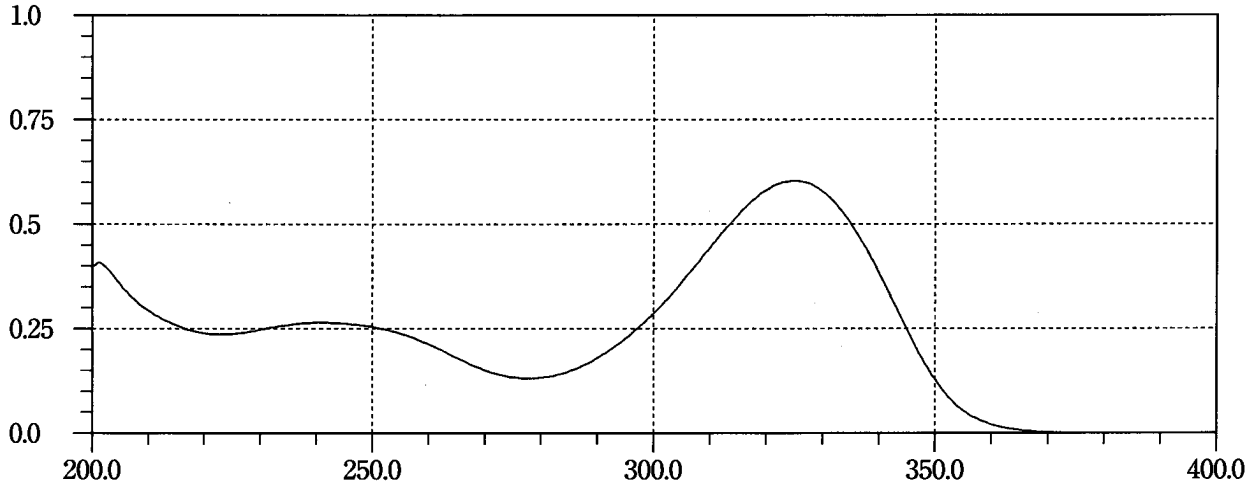
Nafamostat Mesilate



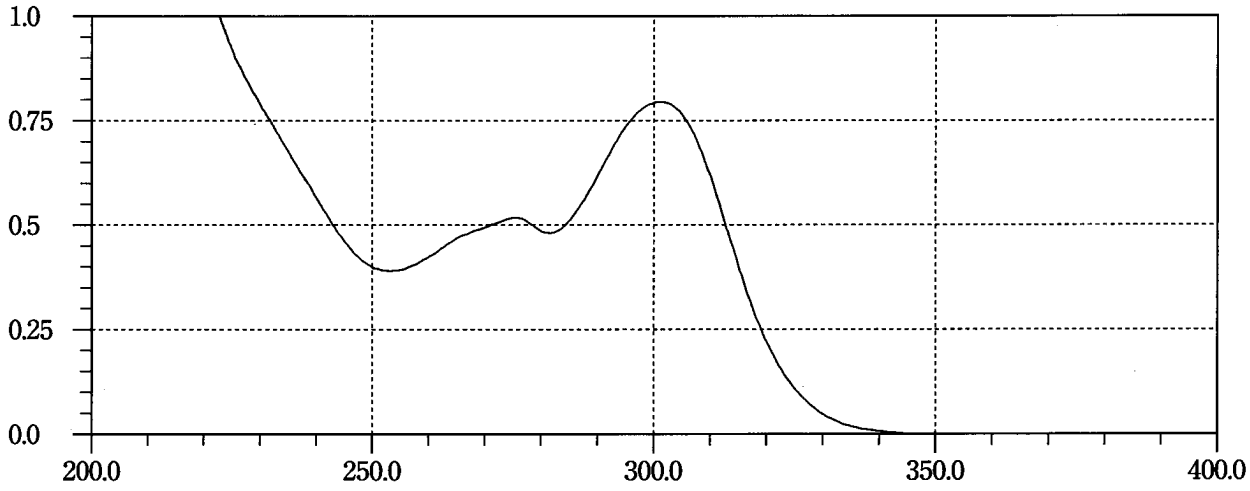
Nicorandil



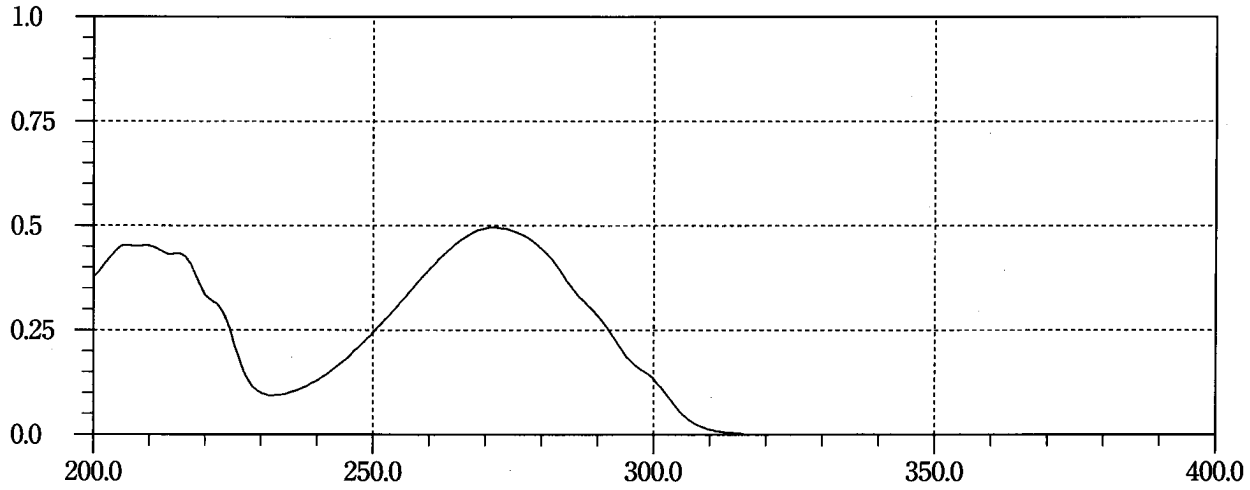
Nizatidine



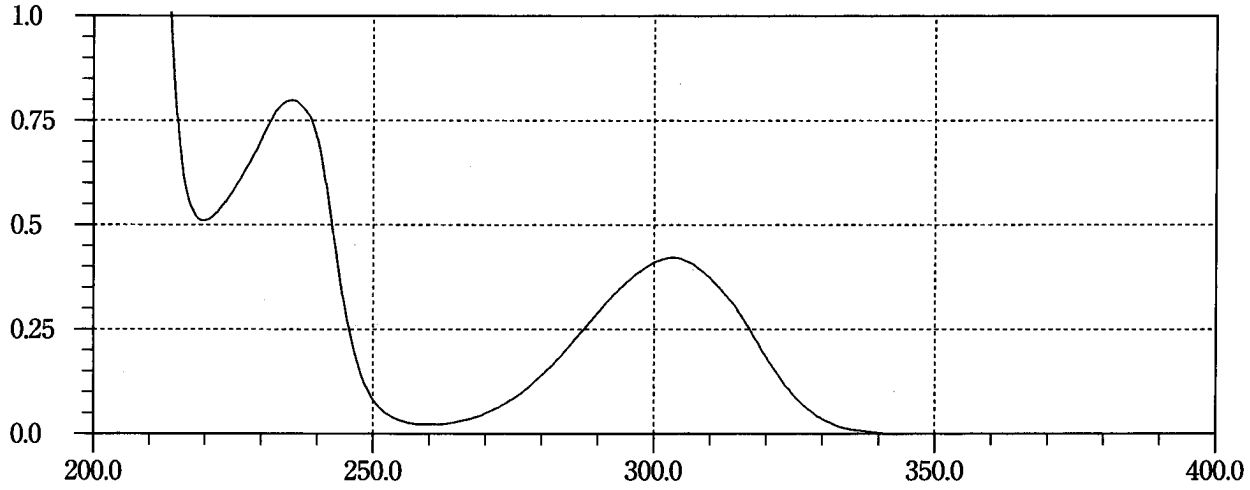
Omeprazole



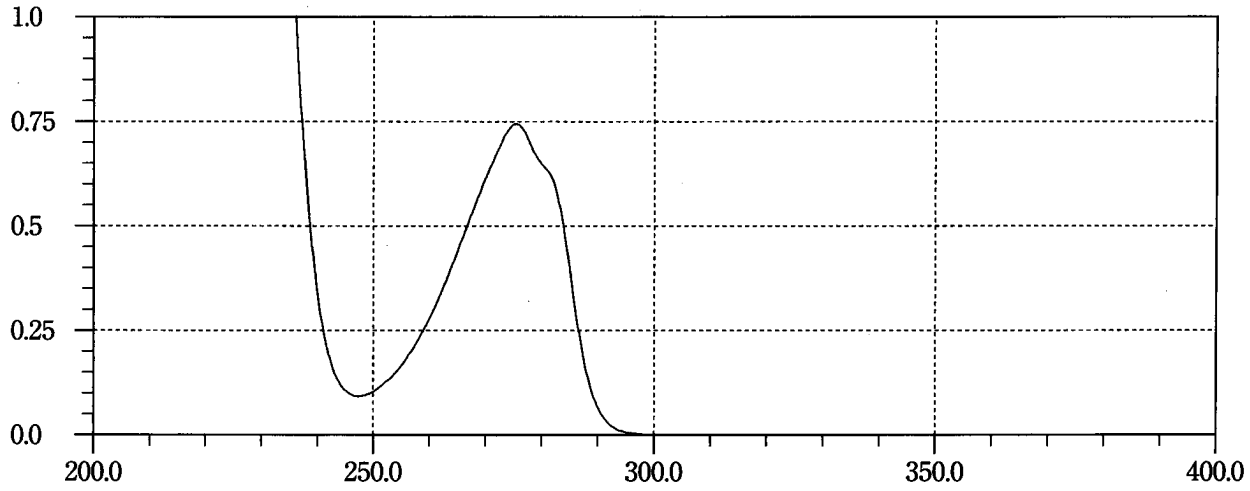
Ozagrel Sodium



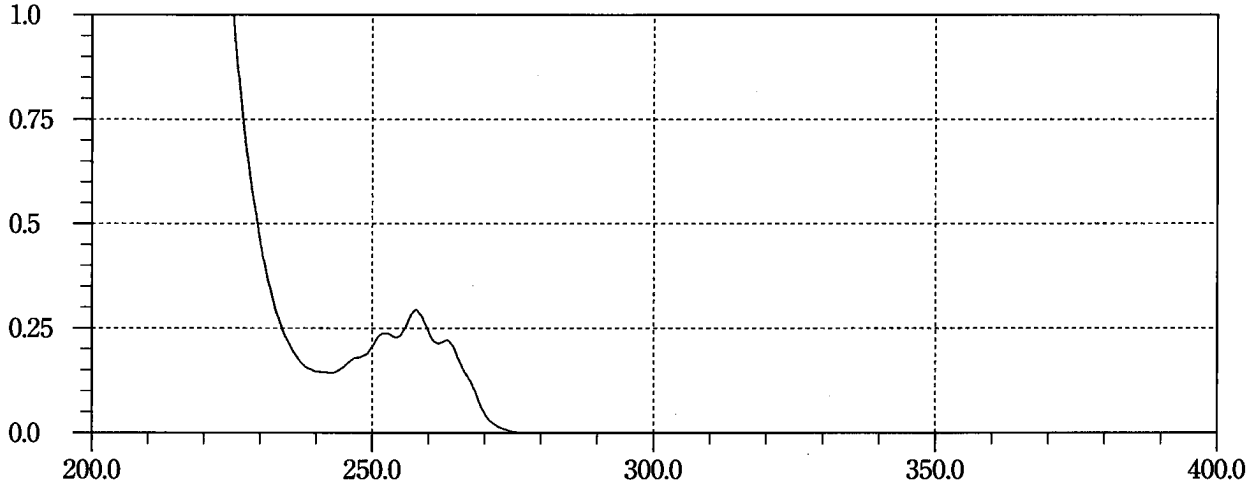
Salicylic Acid



L-Tyrosine



Ubenimex



GENERAL INFORMATION

8. International Harmonization Implemented in the Japanese Pharmacopoeia Fifteenth Edition

Add the following:

May 2005 (Rev.1)

Harmonized items	JP15 (Supplement I)	Remarks
Sodium Starch Glycolate	**Sodium Starch Glycolate**	
Definition	origin, limit of sodium	
Identification A	Identification (1)	
Identification B	Identification (3)	
pH	pH	
Loss on drying	Loss on drying	
Limit of iron	Purity (2) Iron	
Limit of sodium chloride	Purity (4) Sodium chloride	
Limit of sodium glycolate	Purity (3) Sodium glycolate	
Assay	Assay	

Add the following:

June 2006

Harmonized items	JP15 (Supplement I)	Remarks
Hypromellose Phthalate	**Hypromellose Phthalate**	
Definition	origin, limit of carboxybenzoyl	
Packaging and storage	Containers and storage	
Viscosity	Viscosity	
Water	Water	
Residue on ignition	Residue on ignition	
Chloride	Purity (1) Chloride	
Limit of free phthalic acid	Purity (3) Phthalic acid	
Phthalyl content	Assay	

Add the following:

Nov. 2005

Harmonized items	JP15 (Supplement I)	Remarks
Anhydrous Dibasic Calcium Phosphate	**Anhydrous Dibasic Calcium Phosphate**	
Definition	limit of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substance	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfate	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on drying	
Assay	Assay	

Add the following:

Nov. 2005

Harmonized items	JP15 (Supplement I)	Remarks
Dibasic Calcium Phosphate	**Dibasic Calcium Phosphate Hydrate**	
Definition	limit of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substances	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfate	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on drying	
Assay	Assay	

Add the following:

Nov. 2005

Harmonized items	JP15 (Supplement I)	Remarks
Microbiological Examination of Non-sterile Products:	**4.05 Microbiological Examination of Non-sterile Produds**	
Microbial Enumeration Tests	I. Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests	
Introduction	1. Introduction	
General procedures	2. General Procedures	
Enumeration methods	3. Enumeration Methods	
Growth promotion test and suitability of the counting method	4. Growth Promotion Test and Suitability of the Counting Method	

General considerations	4.1. General considerations	
Preparation of test strains	4.2. Preparation of test strains	
Negative control	4.3. Negative control	
Growth promotion of the media	4.4. Growth promotion of the media	
Suitability of the counting method in the presence of product	4.5. Suitability of the counting method in the presence of product	
Results and interpretation	4.6. Results and interpretation	
Testing of products	5. Testing of Products	
Amount used for the test	5.1. Amount used for the test	
Examination of the product	5.2. Examination of the product	
Interpretation of the results	5.3. Interpretation of the results	
Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms	II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms	
Introduction	1. Introduction	
General procedures	2. General Procedures	
Growth promoting and inhibitory properties of the media and suitability of the test	3. Growth Promoting and Inhibitory Properties of the Media and Suitability of the Test	
Preparation of test strains	3.1. Preparation of test strains	
Negative control	3.2. Negative control	
Growth promotion and inhibitory properties of the media	3.3. Growth promotion and inhibitory properties of the media	
Suitability of the test method	3.4. Suitability of the test method	
Testing of products	4. Testing of Products	
Bile-tolerant gram-negative bacteria	4.1. Bile-tolerant gram-negative bacteria	
Escherichia coli	4.2. *Escherichia coli*	
Salmonella	4.3. *Salmonella*	
Pseudomonas aeruginosa	4.4. *Pseudomonas aeruginosa*	
Staphylococcus aureus	4.5. *Staphylococcus aureus*	
Clostridia	4.6. Clostridia	
Candida albicans	4.7. *Candida albicans*	
Recommended solutions and culture media	5. Recommended Solutions and Culture Media	

Add the following:

Nov. 2005

Harmonized items	JP15 (Supplement I)	Remarks
Microbiological Examination of Non-sterile Products:	**General Information** **12. Microbial Attributes of Non-sterile Pharmaceutical Products**	JP's particular description: 1. Definitions 2. Scope

		3. Sampling plan and frequency of testing 4. Microbial control program
Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical use	5. Microbial acceptance criteria for non-sterile pharmaceutical products	
		JP's particular description: Explanation of the microbial acceptance criteria JP's particular description: 6. Acceptance criteria for herbal drugs and herbal drug containing preparations
Table 2. Acceptance Criteria for Microbiological Quality of Non-sterile Substances for Pharmaceutical use	Table 1. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use	
Table 1. Acceptance Criteria for Microbiological Quality of Non-sterile Dosage Forms	Table 2. Acceptance criteria for microbiological quality of non-sterile dosage forms	

Change to read:

Jan. 2000

Harmonized items	JP 15 (Supplement I)	Remarks
Bacterial Endotoxins Test	**4.01 Bacterial Endotoxins Test**	
(Introduction)	(Introduction)	
Apparatus	Apparatus	
Preparation of standard endotoxin stock solution	Preparation of standard endotoxin stock solution	
Preparation of standard endotoxin solution	Preparation of standard endotoxin solution	
Preparation of sample solutions	Preparation of sample solutions	JP's particular description: Addition of the preparation of sample solutions for containers, and deletion of that for medical devices.
Determination of maximum valid dilution	Determination of maximum valid dilution	JP's particular description: Addition of the concentration unit of sample solutions in the case where the endotoxin limit per equivalent is specified.
Gel-clot technique	Gel-clot technique	
(1) Preparatory testing	(1) Preparatory testing	
(i) Test for confirmation of labeled lysate sensitivity	(i) Test for confirmation of labeled lysate sensitivity	
(ii) Test for interfering factors	(ii) Test for interfering factors	
(2) Limit test	(2) Limit test	
(i) Procedure	(i) Procedure	
(ii) Interpretation	(ii) Interpretation	
(3) Assay	(3) Assay	
(i) Procedure	(i) Procedure	

(ii) Calculation and interpretation	(ii) Calculation and interpretation	
Photometric techniques	Photometric techniques	
(1) Turbidimetric techniques	(1) Turbidimetric techniques	
(2) Chromogenic technique	(2) Chromogenic technique	
(3) Preparatory testing	(3) Preparatory testing	
(i) Assurance of criteria for the standard curve	(i) Assurance of criteria for the standard curve	JP's particular description: The test for assurance of criteria for the standard curve must be carried out for each lot of lysate reagent.
(ii) Test for interfering factors	(ii) Test for interfering factors	JP's particular description: Two conditions which the test must meet are specified. Explanation of the test method where the interfering action is found. Explanation of the usual methods for eliminating the interference.
(4) Assay	(4) Assay	
(i) Procedure	(i) Procedure	
(ii) Calculation	(ii) Calculation	JP's particular description: Addition of the alternative requirement to which solution D must meet for valid test.
(iii) Interpretation	(iii) Interpretation	
Reagents, test solutions		deletion
Amebocyte lysate		deletion
Lysate TS		deletion
Water for bacterial endotoxins test (BET)		deletion

Change to read:

Nov. 2005 (Rev. 2)

Harmonized items	JP15 (Supplement I)	Remarks
Anhydrous Lactose	**Anhydrous Lactose**	
Definition	origin	
Specific rotation	Optical rotation	
Clarity and color of solution	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Protein and light-absorbing impurities	Purity (4) Proteins and light absorbing substances	
Loss on drying	Loss on drying	
Water	Water	
Residue on ignition	Residue on ignition	
Content of alpha and beta anomers	Isomer ratio	

Change to read:

12. Microbial Attributes of Non-sterile Pharmaceutical Products

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

The presence of certain micro-organisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations. ◆This chapter provides guidelines for acceptable limits of viable micro-organisms (bacteria and fungi) existing in raw materials and non-sterile pharmaceutical products.◆ Microbial examination of non-sterile products is performed according to the methods given in the Microbial Limit Test *<4.05>* on Microbiological Examination of Non-sterile Products: *Microbial Enumeration Tests* and *Tests for Specified Micro-organisms*. ◆When these tests are carried out, a microbial control program must be established as an important part of the quality management system of the product. Personnel responsible for conducting the tests should have specialized training in microbiology, biosafety measures and in the interpretation of the testing results.◆

◆1. Definitions

1.1 Non-sterile pharmaceutical products: Non-sterile drugs shown in monographs of the JP and non-sterile finished dosage forms.

1.2 Raw materials: All materials, including raw ingredients and excipients, used for the preparation of drugs, except for water and gases.

1.3 Bioburden: Number and type of viable micro-organisms existing in non-sterile pharmaceutical products.

1.4 Action levels: Established bioburden levels that require immediate follow-up and corrective action if they are exceeded.

1.5 Alert levels: Established bioburden levels that give early warning of a potential drift from normal bioburden level, but which are not necessary grounds for definitive corrective action, though they may require follow-up investigation.

1.6 Quality management system: The procedures, operation methods and organizational structure of a manufacturer (including responsibilities, authorities and relationships between these) needed to implement quality management.

2. Scope

In general, the test for total viable aerobic count is not applied to drugs containing viable micro-organisms as an active ingredient.

3. Sampling plan and frequency of testing

3.1 Sampling methods

Microbial contaminants are usually not uniformly distributed throughout the batches of non-sterile pharmaceutical products or raw materials. A biased sampling plan, therefore, cannot be used to estimate the real bioburden in the product. A sampling plan which can properly reflect the status of the product batch should be established on the basis of the bioburden data obtained by retrospective validation and/or concurrent validation. In general, a mixture of samples randomly taken from at least different three portions, almost the same amount for each portion, is used for the tests of the product. When the sampling is difficult in a clean area, special care is required during sampling to avoid introducing microbial contamination into the product or affecting the nature of the product bioburden. If it is confirmed that the product bioburden is stable for a certain period, as in the case of non-aqueous or dried products, it is not necessary to do the tests, immediately after the sampling.

3.2 Testing frequency

The frequency of the tests should be established on the basis of a variety of factors unless otherwise specified. These factors include:

a) Dosage forms of non-sterile pharmaceutical products (usage);
b) Manufacturing processes;
c) Manufacturing frequency;
d) Characteristics of raw materials (natural raw material, synthetic compound, etc.);
e) Batch sizes;
f) Variations in bioburden estimates (changes in batches, seasonal variations, etc.);
g) Changes affecting the product bioburden (changes in manufacturing process, supplier of raw materials, batch number of raw materials, etc.);
h) Others.

In general, the tests may be performed at a high frequency during the initial production of a drug to get information on the microbiological attributes of the product or raw materials used for the production. However, this frequency may be reduced as bioburden data are accumulated through retrospective validation and/or concurrent validation. For example, the tests may be performed at a frequency based on time (e.g., weekly, monthly or seasonally), or on alternate batches.

4. Microbial control program

When the ''Microbial Limit Test *<4.05>*'' is applied to a non-sterile pharmaceutical product, the methods for the recovery, cultivation and estimation of the bioburden from the product must be validated and a ''Microbial control program'' covering the items listed below must be prepared.

a) Subject pharmaceutical name (product name);
b) Frequency of sampling and testing;
c) Sampling methods (including responsible person, quan-

tity, environment, etc. for sampling);
d) Transfer methods of the samples to the testing area (including storage condition until the tests);
e) Treatment of the samples (recovery methods of microbial contaminants);
f) Enumeration of viable micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
g) Detection of specified micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
h) Estimation of the number of and characterization of microbial contaminants;
i) Establishment of ''Microbial acceptance criteria'' (including alert level and action level);
j) Actions to be taken when the levels exceed ''Microbial acceptance criteria'';
k) Persons responsible for the testing and evaluation, etc.;
l) Other necessary items.◆

5. Microbial acceptance criteria for non-sterile pharmaceutical products

By establishing ''Microbial acceptance criteria'' for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC), ◆it is possible to evaluate at the initial processing stage of the product whether the microbiological quality of the raw materials is adequate or not. Furthermore, it is then possible to implement appropriate corrective action as needed to maintain or improve the microbiological quality of the product.◆ The target limits of microbial levels for raw materials (synthetic compounds and minerals) are shown in Table 1.

◆In general, synthetic compounds have low bioburden levels due to the high temperatures, organic solvents, etc., used in their manufacturing processes. Raw materials originated from plants and animals in general have higher bioburdens than synthetic compounds.

The microbial quality of the water used in the processing of active ingredients or non-sterile pharmaceuticals may have a direct effect on the quality of the finished dosage form. This means it is necessary to keep the level of microbial contaminants in the water as low as possible.◆

Acceptance criteria for microbiological quality for non-sterile finished dosage forms are shown in Table 2. ◆These microbial limits are based primarily on the type of dosage form, water activity, and so on. For oral liquids and pharmaceutical products having a high water activity, in general, low microbial acceptance criteria are given.◆

Table 2 includes a list of specified micro-organisms for which acceptance criteria are set. The list is not necessarily exhaustive and for a given preparation it may be necessary to test for other micro-organisms depending on the nature of the starting materials and the manufacturing process.

If it has been shown that none of the prescribed tests will allow valid enumeration of micro-organisms at the level

Table 1. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use

	Total Aerobic Microbial Count (CFU/g or CFU/mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)
Substances for pharmaceutical use	10^3	10^2

Table 2. Acceptance criteria for microbiological quality of non-sterile dosage forms

Route of administration	Total Aerobic Microbial Count (CFU/g or CFU/mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)	Specified Micro-organism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	—
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)

Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (more rigorous requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant gram-negative bacteria (1g or 1 mL)

prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

In addition to the micro-organisms listed in Table 2, the significance of other micro-organisms recovered should be evaluated in terms of:

- the use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
- the nature of the product: does the product support growth, does it have adequate antimicrobial preservation?
- the method of application;
- the intended recipient: risk may differ for neonates, infants, the debilitated;
- use of immunosuppressive agents, corticosteroids;
- presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and the interpretation of microbiological data.

For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:
—10^1 CFU: maximum acceptable count = 20,
—10^2 CFU: maximum acceptable count = 200,
—10^3 CFU: maximum acceptable count = 2000, and so forth.

◆**6. Acceptance criteria for herbal drugs**

Target limits of microbial contamination for herbal drugs and herbal drug containing preparations are shown in Table 3. Category 1 indicates herbal drugs and their preparations to which boiling water is added before use, and category 2 indicates other herbal drugs and their preparations. In this guideline, enterobacteria and other gram-negative bacteria, *Escherichia coli, Salmonella*, and *Staphylococcus aureus* are mentioned as specified micro-organisms, but other micro-organisms such as certain species of *Bacillus cereus*, Clostridia, *Pseudomonas, Burkholderia, Asperigillus* and *Enterobacter* species are also necessary to be tested depending on the origin of the herbal drug raw materials or the preparation method of the preparations.◆

◆**Table 3.** Acceptance criteria for herbal drugs and their preparations

Micro-organisms	Category 1 (CFU/g or CFU/mL)	Category 2 (CFU/g or CFU/mL)
Aerobic bacteria	10^7	10^5
Molds and yeasts	10^4	10^3
Enterobacteria and other gram-negative bacteria	※	10^3
Escherichia coli	10^2	Not detected
Salmonella	Not detected	Not detected
Staphylococcus aureus	※	※

※ : The limits are not specified.◆

21. Quality Control of Water for Pharmaceutical Use

Change the 3.4.1 Media and Incubation Conditions to read:

3.4.1 Media and Incubation Conditions

There are many mesophilic bacteria of heterotrophic type that are adaptable to poor nutrient water environments. In many pharmaceutical water systems, heterotrophic bacteria may form bio-films and cause water quality deterioration. It, therefore, is useful to monitor the water quality by use of R2A Agar Medium, which is excellent for growing bacteria of oligotrophic type. On the other hand, in routine microbial monitoring, an approach that identifies the trend in microbiological quality change is widely employed; a standard agar plate is used for counting the total number of viable microorganisms capable of proliferating at 30–35°C in a comparatively short period of time.

Table 2 shows examples of measurement methods, minimum sample sizes, media, and incubation periods for estimating viable counts.

The media shown in Table 2 are as follows.

Table 2. Methods for Assessment of Viable Counts in Pharmaceutical Water

Method	Pharmaceutical Water		
	Water	Purified Water in Bulk	Water for Injection in Bulk
Measurement Method	Pour Plate Method or Membrane Filtration	Pour Plate Method or Membrane Filtration	Membrane Filtration
Minimum Sample Size	1.0 mL	1.0 mL	100 mL
Media	Standard Agar Medium	R2A Agar Medium, Standard Agar Medium	R2A Agar Medium, Standard Agar Medium
Incubation Period	Standard Agar Medium: 48 – 72 hours (or longer)	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)
Incubation Temperature	Standard Agar Medium: 30 – 35°C	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C

Standard Agar Medium

Casein peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9–7.1.

R2A Agar Medium

Peptone (casein and animal tissue)	0.5 g
Casamino acid	0.5 g
Yeast extract	0.5 g
Sodium pyruvate	0.3 g
Glucose	0.5 g
Magnesium sulfate heptahydrate	0.05 g
Soluble starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Agar	15 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.3.

For the ingredients, which are not specified in the Japanese Pharmacopoeia, use the following reagents.

Casamino acid Prepared for microbial test, by the acid hydrolysis of casein.

Loss on drying ⟨*2.41*⟩: Not more than 8% (0.5 g, 105°C, constant mass).

Residue on ignition ⟨*2.44*⟩: Not more than 55% (0.5 g).

Nitrogen content ⟨*1.08*⟩: Not less than 7% (105°C, constant mass, after drying).

Sodium pyruvate $CH_3COCOONa$ White to pale yellow crystalline powder. Freely soluble in water, and slightly soluble in ethanol (99.5) and in acetone.

Identification (1) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1710 cm^{-1}, 1630 cm^{-1}, 1410 cm^{-1}, 1360 cm^{-1}, 1190 cm^{-1}, 1020 cm^{-1}, 980 cm^{-1}, 830 cm^{-1}, 750 cm^{-1}, 630 cm^{-1} and 430 cm^{-1}.

(2) A solution (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Content: not less than 97.0%. Assay—Dissolve about 0.4 g of sodium pyruvate, accurately weighed, in 200 mL of water. Transfer 20 mL of this solution into a ground joint iodine bottle, and cool to 10°C or lower. Add 40 mL of 0.05 mol/L iodine VS and 20 mL of 4 mol/L sodium hydroxide solution, then allow to stand in a dark place for 2 hours, and add 15 mL of diluted sulfuric acid (1 in 6). Titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 1.854 mg of $C_3H_3NaO_3$

Add the following:

31. Purity Tests on Crude Drugs Using Genetic Information

The first step in the quality assurance of natural products is the use of raw materials from the right part of the right

origin (the right source). Therefore, it is clearly stated in Article 4 of the General Rules For Crude Drugs that the source of a crude drug is the approval or rejection criteria. There are various methods for differentiating the sources of crude drugs, such as morphological methods, organoleptic tests, and chemical methods, and appropriate methods for each are described in the individual monographs. Morphological methods, organoleptic tests, and chemical methods are discrimination methods for species that are based on the expression characteristics of the crude drugs. On the other hand, together with recent progress in molecular biology techniques and the accumulation of genetic information on plants, methods for differentiating species have been established by confirming the genotype of a crude drug. Methods such as these are different from morphological and other methods that differentiate based on phenotype in that they are not affected by the environment. Also, the methods have several advantages, such as specialized expertise and skill for classification are not needed, and objective results are easily obtained.

The evolution of living organisms is accomplished by genetic mutation, and differences among the base sequences of genes of closely related species reflect the strain relationships between the species. Based on this theory, in recent years methods that classify species phylogenetically using the base sequence of rDNA that codes for ribosomal RNA (rRNA) on the nuclear genome have been adopted. In the same way, the base sequence of this rDNA is also the most often used in the classification of higher plants using the genotype. In particular, it has become very easy to classify closely related species using the intergenic transcriber space (ITS) region of the rDNA region, since by comparison with the coded gene region base substitution is easily undertaken. Furthermore, since the genes on the nuclear genome originate from the parents, an advantage is interspecies hybrids can be confirmed. Higher plants also have mitochondrial genes and chloroplastic genes. Although the genes on these genomes are also often used for classification, interspecies hybrids cannot be confirmed because the genes are normally monogenic.

The methods presented here have been developed based on the reported identification methods of Atractylodes Lancea Rhizome and Atractylodes Rhizome (Y. Guo, *et al*, *J. Nat. Med*. 60, 149–156, 2006) utilizing the gene sequence of the ITS of rDNA. Cooperative studies on the validation of purity tests related to Atractylodes Lancea Rhizome in Atractylodes Rhizome, have been completed. The plant sources for Atractylodes Lancea Rhizome stipulated in the individual monographs are *Atractylodes lancea* De Candolle or *A. chinensis* Koidzumi (*Compositae*), while those for Atractylodes Rhizome are *A. japonica* Koidzumi ex Kitamura or *A. ovata* De Candolle (*Compositae*). The approval or rejection of the source of Atractylodes Lancea Rhizome is, in principle, determined by the description of the crude drug, including microscopy, while that of Atractylodes Rhizome is determined by the description of the crude drug, including microscopy, together with color reaction, which is an identification test. In the manuscript, it was shown that these 4 types of plants can be clearly classified by comparing the base sequences of the ITS mentioned above, and that the species can be easily classified without performing base sequence analysis by performing PCR using a species specific primer set and determining the presence or absence of an amplification band.

In cooperative studies, the degree of simplicity of a study is given maximum consideration. We examined methods that observe PCR amplification bands using species specific primer sets and do not involve base sequence analyses. Test methods based on PCR which uses species specific primer sets are analytical methods with very high degrees of sensitivity. Therefore, when using them as identification tests for powdered crude drugs, amplification bands can be observed even if the vast majority of the crude drug for analysis is not suitable and there is only a minute amount of powder from a crude drug derived from a suitable plant. Consequently, in identification tests, either a cut or a whole crude drug must be used, as long as one is careful to avoid contamination by powder originating from other crude drugs. On the other hand, when used as a purity test, the form of the crude drug is irrelevant as long as the gene amplification is performed properly and the target gene is not polymorphic, so if amplification bands of an inappropriate plant are confirmed in the purity test, regardless of the form of the crude drug, it becomes clear there is contamination by an inappropriate crude drug.

The methods shown here are reference information and at the present stage results obtained using the methods do not affect the approval or rejection of the crude drug in each monograph. Furthermore, by performing the sequence outlined in the previous paper, it goes without saying that a more accurate decision concerning the source species can be made.

DNA Amplification Equipment

DNA amplification equipment is used to amplify the DNA which is extracted from a crude drug and then purified. Since there are slight differences in the methods of temperature control, and so on depending on the equipment used, there may be differences in the intensity, etc. of the PCR amplification bands even if PCR is carried out under the stipulated conditions. Therefore, when judging results based solely on the presence or absence of PCR amplification bands, confirm that only proper amplification bands are obtained when performing PCR using DNA obtained using samples confirmed beforehand to be the source species. If proper amplification bands are not obtained, the PCR temperature conditions should be slightly adjusted.

Procedure

The following is a sample procedure.

1. Preparation of template DNA

Crude drugs are different from fresh plants in that they are dried products and a certain amount of time has passed since they were harvested. Therefore, in many cases the DNA has undergone fragmentation. Furthermore, various substances that can block or interfere with the PCR reaction may be present in the plant. For these reasons, the extraction

and purification of template DNA is the process that should receive the greatest amount of attention. In the case of Atractylodes crude drugs, the periderm should be removed using a clean scalpel or other clean instrument before pulverizing the sample because very often there are inhibitory substances present in the periderm.

There are various methods with which to extract and purify DNA from the samples. It is recommended that commercially available DNA extraction kits be used if one takes into consideration their advantages of not using any noxious reagents and not requiring any complicated purification procedures. In this case, attention should be paid to the final amount (concentration) of DNA obtained, and the amount of initial sample and the volume of liquid to elute the DNA need to be controlled. When extraction and purification are performed using silica gel membrane type kits stipulated in notifications (Notification No. 110, Director of Food Health Department, March 2001; Partial Amendment: Notification No. 0629002, 2.2.1.2, Director of Food Safety Department, June 2006) related to inspection methods for recombinant DNA foods, it is appropriate to use 200 mg of sample, 1 mL of AP1 buffer solution, 2 μL of RNase A, and 325 μL of AP2 buffer solution. Also, the most important things are that the supernatant loaded on the first column is clear and that there is no need to load 1 mL unreasonably. Furthermore, 50 μL is an appropriate volume used in the final elution of the DNA, and normally the initial eluate is used as the DNA sample stock solution.

2. Confirmation of purity of DNA in DNA sample stock solution and assay of DNA

The purity of the DNA in the stock solution can be confirmed by the $OD_{260\,nm}/OD_{280\,nm}$ ratio using a spectrophotometer. A ratio of 1.5 indicates that the DNA has been adequately purified. The amount of DNA is calculated using 1 $OD_{260\,nm} = 50\,\mu g/mL$. The measurement mentioned above is performed using appropriately diluted DNA sample stock solution. Based on the results obtained, dilute with water to the concentration needed for the subsequent PCR reactions, use this solution as the DNA sample solution, pipet into micro test tubes, and if necessary store frozen at not over $-20°C$. The pipeted DNA sample is used immediately after thawing and any remaining solution should be discarded and not refrozen. If the concentration of the DNA sample stock solution does not reach the concentration stipulated in PCR, it is used as a DNA sample solution.

3. PCR

When a commercially available enzyme is used with the qualitative PCR method mentioned in the above notification (Notification No. 0629002, 2.1.3.1.1, Director of Food Safety Department), to a solution consisting of 2.5 μL of the PCR buffer solution containing magnesium that comes with the enzyme, dNTP (0.2 mmol/L) that also comes with the enzyme, 5' and 3' primer (0.4 μmol/L), and Taq DNA polymerase (1.25 units), add 5 μL of 10 ng/μL DNA sample solution (50 ng of DNA) on ice. It is appropriate to perform the reaction at a total volume of 25 μL. When conducting purity tests on Atractylodes Lancea Rhizome in Atractylodes Rhizome, the primer sets used are C and D (C is positive with *A. lancea*, D is positive with A. *chinensis*) as described in the paper mentioned above (*J. Nat. Med.* 60, 149–156, 2006), however, when a combination of primer A and B is used, it is possible to confirm the source species of each of the respective specimens. In order to confirm that the DNA has been extracted correctly, the reaction solution containing the positive control primer (Pf and Pr) as shown below should be prepared. In addition, the negative control solutions which are not containing DNA sample or either of the primer sets should be prepared and simultaneously conduct PCR.

Pf: 5'-CAT TGT CGA AGC CTG CAC AGC A-3'
Pr: 5'-CGA TGC GTG AGC CGA GAT ATC C-3'

The PCR reaction is performed under the following conditions. After starting the reaction at 95°C for 10 minutes, followed by one cycle of 0.5 minutes at 95°C and 0.75 minutes at 68°C (69°C only when using the primer set C), and 30 cycles of PCR amplification. Then terminate reaction at 72°C for 7 minutes, store at 4°C, and use the reaction solution obtained as the PCR amplification reaction solution.

4. Gel electrophoresis

After completion of the reaction, mix 5 μL of the PCR amplification reaction solution with an appropriate volume of gel loading buffer solution, add the mixture to the wells of 2% agarose gel, and then perform electrophoresis using 1-fold TAE buffer solution (refer to General Information, Rapid Identification of Microorganisms Based on Molecular Biological Method). Run in parallel an appropriate DNA molecular mass standard. Electrophoresis is terminated when the bromophenol blue dye in the gel loading buffer has advanced to a point corresponding to 1/2 to 2/3 the length of the gel.

5. Detection and evaluation of PCR products

Counterstain the gel after electrophoresis when not using gel stained in advance with ethidium bromide. Place the gel that has undergone electrophoresis and staining in a gel image analyzer, irradiate with ultraviolet light (312 nm), and determine its electrophoresis pattern. Compare this to the DNA molecular mass standard and determine the absence or presence of the target amplification band. In the case of purity tests on Atractylodes Lancea Rhizome in Atractylodes Rhizome, first confirm a 305 bp band with the reaction solution to which the positive control primer set has been added, and confirm there are no bands in a solution with no primer sets and a solution with no DNA sample solution. Next, if a 226 bp band is confirmed when the primer set C is added or if a 200 bp band is confirmed when the primer set D is added, the sample is judged to be Atractylodes Lancea Rhizome (in the case of cut crude drug, contamination of Atractylodes Lancea Rhizome is observed) and it is rejected. The sample is judged not to be Atractylodes Lancea Rhizome (in the case of cut crude drug, there is no contamination of Atractylodes Lancea Rhizome) and the purity test is acceptable if a 305 bp band is confirmed with the positive control primer set, bands are not observed in reaction solution without primer and reaction solution

without DNA sample solution, and a 226 bp band is not observed with the primer set C and a 200 bp band is not observed with the primer set D. If a band is not observed with the positive control primer, it is to be concluded that the DNA extraction failed and the procedure should be started over again from the DNA extraction step. If bands are confirmed in reaction solutions without primer sets or without DNA sample solution, it should be assumed that there was an error in the PCR procedure and therefore the procedure should be repeated again from the step 3. PCR.

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