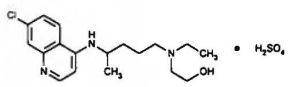
chloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, using sodium carbonate TS in place of 1 N sodium hydroxide, beginning with "Transfer the liquid to a separator": the Ophthalmic Solution meets the requirements of the test.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 4.2 and 6.0.

Assay—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 100 mg of hydroxyamphetamine hydrobromide, to a 125-mL separator. Wash the solution with 15 mL of chloroform, and discard the washing. Rinse the stopper and the mouth of the separator with a few drops of water. Add 1.05 g of sodium bicarbonate, preventing it from coming in contact with the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-mL syringe, rapidly inject 0.5 mL of acetic anhydride directly into the contents of the separator. Immediately insert the stopper in the separator, and shake vigorously until the evolution of carbon dioxide has ceased (7 to 10 minutes), releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes, and extract the solution with five 10-mL portions of chloroform, filtering each extract through a pledget of cotton, previously washed with chloroform, into a tared 100-mL beaker. Evaporate the combined chloroform extracts on a steam bath in a current of air or stream of nitrogen to dryness. Dry the residue at 80° for 90 minutes, cool in a desiccator, and weigh. The weight of the diacetylhydroxyamphetamine so obtained, multiplied by 0.9866, represents the weight of $C_9H_{13}NO \cdot HBr$ in the volume of Ophthalmic Solution taken.

Hydroxychloroquine Sulfate



$C_{18}H_{26}ClN_3O \cdot H_2SO_4$ 433.95

Ethanol, 2-[[4-[(7-chloro-4-quinolinyl)amino]pentyl]ethyl]amino-, (±)-, sulfate (1 : 1) (salt).

(±)-2-[[4-[(7-Chloro-4-quinolyl)amino]pentyl]ethylamino]ethanol sulfate (1 : 1) (salt) [*747-36-4*].

» Hydroxychloroquine Sulfate contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{26}ClN_3O \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Hydroxychloroquine Sulfate RS.*

Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: dilute hydrochloric acid (1 in 100).

B: *Infrared Absorption* ⟨197K⟩.

C: A solution (1 in 100) responds to the tests for *Sulfate* ⟨191⟩.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 2.0% of its weight.

Ordinary impurities ⟨466⟩—

Test solution: 10% water in methanol.

Standard solution: 10% water in methanol.

Eluant: a mixture of alcohol, water, and ammonium hydroxide (80 : 16 : 4).

Visualization: 1.

Organic volatile impurities, *Method I* ⟨467⟩: meets the requirements.

Assay—Dissolve about 100 mg of Hydroxychloroquine Sulfate, accurately weighed, in about 5 mL of water, and dilute quantitatively and stepwise with dilute hydrochloric acid (1 in 100) to obtain a solution containing about 10 µg per mL. Similarly prepare a Standard solution of USP Hydroxychloroquine Sulfate RS. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 343 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 100) as the blank. Calculate the quantity, in mg, of $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ in the portion of Hydroxychloroquine Sulfate taken by the formula:

$$10C(A_U / A_S),$$

in which C is the concentration, in µg per mL, of USP Hydroxychloroquine Sulfate RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Hydroxychloroquine Sulfate and the Standard solution, respectively.

Hydroxychloroquine Sulfate Tablets

» Hydroxychloroquine Sulfate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of hydroxychloroquine sulfate ($C_{18}H_{26}ClN_3O \cdot H_2SO_4$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Hydroxychloroquine Sulfate RS.*

Identification—

A: Triturate a quantity of finely powdered Tablets, equivalent to about 1 g of hydroxychloroquine sulfate, with 50 mL of water, and filter: the clear filtrate so obtained meets the requirements for *Identification* tests *B* and *C*.

B: It meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

C: A solution (1 in 100) meets the requirements of the tests for *Sulfate* ⟨191⟩.

D: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution ⟨711⟩—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 343 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Hydroxychloroquine Sulfate RS in the same medium.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{18}H_{26}ClN_3O \cdot H_2SO_4$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—To 800 mL of water, add 100 mL of methanol, 100 mL of acetonitrile, 2.0 mL of phosphoric acid, and 96 mg of sodium 1-pentanesulfonate, mix, and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Solvent mixture—Prepare a mixture of methanol and water (1 : 1).

Standard preparation—Dissolve an accurately weighed quantity of USP Hydroxychloroquine Sulfate RS in *Solvent mixture*, dilute quantitatively with *Solvent mixture*, and mix to obtain *Solution A* having a known concentration of about 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain the *Standard preparation* having a known concentration of about 0.05 mg per mL.

Resolution solution—Prepare a solution of chloroquine phosphate in methanol having a concentration of 1 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Solution A*, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of hydroxychloroquine sulfate to a 200-mL volumetric flask, add about 150 mL of *Solvent mixture*, and mix. Sonicate, with intermittent shaking, for about 15 minutes, and cool to

Heavy metals, *Method II* ⟨231⟩: 0.001%, 1 mL of hydroxylamine hydrochloride solution (1 in 5) being added to the solution of the residue.

Organic volatile impurities, *Method IV* ⟨467⟩: meets the requirements.

Assay—*[Caution—Hydriodic acid and its reaction byproducts are highly toxic. Perform all steps of the Assay preparation and the Standard preparation in a properly functioning hood. Specific safety practices to be followed are to be identified to the analyst performing this test.]*

Hydriodic acid—Use a reagent having a specific gravity of at least 1.69, equivalent to 55% HI.

Internal standard solution—Transfer about 2.5 g of toluene, accurately weighed, to a 100-mL volumetric flask containing 10 mL of *o*-xylene, dilute with *o*-xylene to volume, and mix.

Standard preparation—Into a suitable serum vial weigh about 135 mg of adipic acid and 4.0 mL of *Hydriodic acid*, pipet 4 mL of *Internal standard solution* into the vial, and close the vial securely with a suitable septum stopper. Weigh the vial and contents accurately, add 30 µL of isopropyl iodide through the septum with a syringe, again weigh, and calculate the weight of isopropyl iodide added, by difference. Add 90 µL of methyl iodide similarly, again weigh, and calculate the weight of methyl iodide added, by difference. Shake, and allow the layers to separate.

Assay preparation—Transfer about 0.065 g of dried Hypromellose, accurately weighed, to a 5-mL thick-walled reaction vial equipped with a pressure-tight septum-type closure, add an amount of adipic acid equal to the weight of the test specimen, and pipet 2 mL of *Internal standard* into the vial. Cautiously pipet 2 mL of *Hydriodic acid* into the mixture, immediately cap the vial tightly, and weigh accurately. Mix the contents of the vial continuously, while heating at 150° for 60 minutes. Allow the vial to cool for about 45 minutes, and again weigh. If the weight loss is greater than 10 mg, discard the mixture, and prepare another *Assay preparation*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a thermal conductivity detector and a 4-mm × 1.8-m glass column packed with 20% liquid phase G28 on 100- to 120-mesh support S1C that is not silanized. Helium is used as the carrier gas and the temperature of the column is maintained at 130°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0, 2.2, 3.6, and 8.0 for methyl iodide, isopropyl iodide, toluene, and *o*-xylene, respectively; and the resolution, *R*, between toluene and isopropyl iodide is not less than 2.0.

Calibration—Inject about 2 µL of the upper layer of the *Standard preparation* into the gas chromatograph, and record the chromatogram. Calculate the relative response factor, F_M, of equal weights of toluene and methyl iodide taken by the formula:

$$Q_M / R_{SM},$$

in which Q_M is the quantity ratio of methyl iodide to toluene in the *Standard preparation*, and R_{SM} is the peak area ratio of methyl iodide to toluene obtained from the *Standard preparation*. Similarly, calculate the relative response factor, F_I, of equal weights of toluene and isopropyl iodide taken by the formula:

$$Q_I / R_{SI},$$

in which Q_I is the quantity ratio of isopropyl iodide to toluene in the *Standard preparation*, and R_{SI} is the peak area ratio of isopropyl iodide to toluene obtained from the *Standard preparation*.

Procedure—Inject about 2 µL of the upper layer of the *Assay preparation* into the gas chromatograph, and record the chromatogram. Calculate the percentage of methoxy ($-OCH_3$) in the Hypromellose taken by the formula:

$$2(31/142)F_M R_{UM}(W_T / W_U),$$

in which 31/142 is the ratio of the formula weights of methoxy and methyl iodide; F_M is defined under *Calibration;* R_{UM} is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay preparation;* W_T is the weight, in g, of toluene in the *Internal standard solution;* and W_U is the weight, in g, of Hypromellose taken for the *Assay*. Similarly, calculate the percentage of hydroxypropoxy ($-OCH_2CHOHCH_3$) in the Hypromellose taken by the formula:

$$2(75/170)F_I R_{UI}(W_T / W_U),$$

in which 75/170 is the ratio of the formula weights of hydroxypropoxy and isopropyl iodide; F_I is defined under *Calibration;* R_{UI} is the ratio of the area of the isopropyl iodide peak to that of the toluene peak obtained from the *Assay preparation;* W_T is the weight, in g, of toluene in the *Internal standard solution;* and W_U is the weight, in g, of Hypromellose taken for the *Assay*.

Hypromellose Ophthalmic Solution

» Hypromellose Ophthalmic Solution is a sterile solution of Hypromellose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of Hypromellose (hydroxypropyl methylcellulose). It may contain suitable antimicrobial, buffering, and stabilizing agents.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Hydroxypropyl Methylcellulose RS.*

Identification—

A: It meets the requirements of *Identification* test *C* under *Hypromellose*.

B: Heat 5 mL of Ophthalmic Solution in a test tube over a low flame: the warm solution turns cloudy but clears upon chilling.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 6.0 and 7.8.

Assay—

Standard preparation—Dissolve a suitable quantity of USP Hydroxypropyl Methylcellulose RS, accurately weighed, in water and dilute quantitatively with water to obtain a solution having a known concentration of about 100 µg per mL.

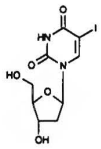
Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively with water to obtain a solution having an equivalent concentration of about 100 µg of hypromellose per mL.

Procedure—Pipet 2 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide a blank, into separate, glass-stoppered test tubes. To each tube add 5.0 mL of diphenylamine solution (prepared by dissolving 3.75 g of colorless diphenylamine crystals in 150 mL of glacial acetic acid and diluting the solution with 90 mL of hydrochloric acid), mix, and immediately insert the tubes into an oil bath at 105° to 110° for 30 minutes, the temperature being kept uniform within 0.1° during heating. Remove the tubes, and place them in an ice-water bath for 10 minutes or until thoroughly cool. At room temperature and using a suitable spectrophotometer, concomitantly determine the absorbances of the solutions from the *Standard preparation* and the *Assay preparation* at 635 nm, using the water solution as the blank. Calculate the quantity, in mg, of hypromellose in each mL of the Ophthalmic Solution taken by the formula:

$$0.001C(d/V)(A_U / A_S),$$

in which C is the concentration, in µg per mL, of USP Hydroxypropyl Methylcellulose RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken; d is the dilution fold of V used to obtain the *Assay preparation;* and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Idoxuridine



$C_9H_{11}IN_2O_5$ 354.10

Uridine, 2′-deoxy-5-iodo-.

2′-Deoxy-5-iodouridine [*54-42-2*].

Idoxuridine contains not less than 98.0 percent and not more than 101.0 percent of $C_9H_{11}IN_2O_5$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Idoxuridine RS.*

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 35 µg per mL.

Medium: pH 12.0 buffer (prepared from 7.46 g of potassium chloride and 24 mL of 1 N sodium hydroxide dissolved in 2000 mL of water).

Absorptivities at 279 nm, calculated on the dried basis for the test sample only, do not differ by more than 2.0%.

Loss on drying ⟨731⟩—Dry about 500 mg, accurately weighed, in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.

Assay—Dissolve about 250 mg of Idoxuridine, accurately weighed, in 70 mL of dimethylformamide that previously has been neutralized with 0.1 N sodium methoxide in toluene VS, a solution of 300 mg of thymol blue in 100 mL of methanol being used as the indicator. Titrate with 0.1 N sodium methoxide in toluene VS to a blue endpoint, taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 35.41 mg of $C_9H_{11}IN_2O_5$.

Idoxuridine Ophthalmic Ointment

» Idoxuridine Ophthalmic Ointment is Idoxuridine in a petrolatum base. It contains not less than 0.45 percent and not more than 0.55 percent of $C_9H_{11}IN_2O_5$. It is sterile.

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes in a cool place.

USP Reference standards ⟨11⟩—*USP Idoxuridine RS.*

Identification—The UV absorption spectrum of the solution from the Ophthalmic Ointment employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as those of the *Standard preparation* prepared for the *Assay.*

Sterility ⟨71⟩: meets the requirements.

Metal particles—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* ⟨751⟩.

Assay—

Chromatographic column—Mix 4 g of chromatographic siliceous earth with 4 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Transfer to a 19- × 250-mm chromatographic tube (see *Chromatography* ⟨621⟩) that contains a pledget of glass wool and is fitted with a stopcock at the bottom. Tamp gently to compress to a uniform mass.

Standard preparation—Transfer about 25 mg of USP Idoxuridine RS, accurately weighed, to a 50-mL volumetric flask, add methanol to volume, and mix. Dilute 5.0 mL of this solution with a mixture of 1 volume of butyl alcohol and 5 volumes of chloroform to 100.0 mL, and mix.

Assay preparation—Mix 4 g of chromatographic siliceous earth with 2 mL of 0.1 N hydrochloric acid in a glass mortar until the mixture is fluffy. Add a quantity of Ophthalmic Ointment, equivalent to about 5 mg of idoxuridine and accurately weighed, to the mixture, and mix.

Procedure—Transfer the *Assay preparation* to the prepared *Chromatographic column.* Transfer 2 g of chromatographic siliceous earth and 2 mL of 0.1 N hydrochloric acid to the glass mortar, and mix until fluffy, using this material to rinse the mortar and pick up any remaining Ophthalmic Ointment. Transfer about half of this mixture to the tube, and tamp gently until the column appears uniform. Transfer the remaining portion to the *Chromatographic column*, and tamp as before. Wipe the walls of the mortar with a small pledget of glass wool, and insert the pledget in the top of the column. Pass 50 mL of chloroform through the column at a flow rate of approximately 1 mL per minute, and discard the chloroform. Elute with about 200 mL of a mixture of 1 volume of butyl alcohol and 5 volumes of chloroform at the same flow rate, discarding the first 20 mL of the eluate. Collect the remainder of the eluate in a 200-mL volumetric flask, dilute with the eluting solvent to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard preparation* in 1-cm cells at 320 nm and at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer, using a mixture of butyl alcohol and chloroform as the blank. Calculate the quantity, in mg, of $C_9H_{11}IN_2O_5$ in the Ophthalmic Ointment taken by the formula:

$$0.2C(A_{283} - A_{320})_U/(A_{283} - A_{320})_S,$$

in which C is the concentration, in µg per mL, of USP Idoxuridine RS in the *Standard preparation;* and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Ophthalmic Ointment (U) and the *Standard preparation* (S), respectively.

Idoxuridine Ophthalmic Solution

» Idoxuridine Ophthalmic Solution is a sterile, aqueous solution of Idoxuridine. It contains not less than 0.09 percent and not more than 0.11 percent of $C_9H_{11}IN_2O_5$. It may contain suitable buffers, stabilizers, and antimicrobial agents.

Packaging and storage—Preserve in tight, light-resistant containers in a cold place.

USP Reference standards ⟨11⟩—*USP Idoxuridine RS.*

Identification—The UV absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of the *Standard preparation* prepared for the *Assay.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 4.5 and 7.0.

Assay—

Chromatographic column and *Standard preparation*—Prepare as directed in the *Assay* under *Idoxuridine Ophthalmic Ointment.*

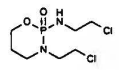
Assay preparation—Mix an accurately measured volume of Ophthalmic Solution, equivalent to about 5 mg of idoxuridine, with 3 g of chromatographic siliceous earth in a glass mortar until the mixture is fluffy.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Idoxuridine Ophthalmic Ointment*, omitting the treatment of the column with 50 mL of chloroform. Calculate the quantity, in mg, of $C_9H_{11}IN_2O_5$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.2C(A_{283} - A_{320})_U / V(A_{283} - A_{320})_S,$$

in which C is the concentration, in µg per mL, of USP Idoxuridine RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and the parenthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the Solution (U) and the *Standard preparation* (S), respectively.

Ifosfamide



$C_7H_{15}Cl_2N_2O_2P$ 261.09

2*H*-1,3,2-Oxazaphosphorin-2-amine,*N*,3-bis(2-chloroethyl)tetrahydro-, 2-oxide.

3-(2-Chloroethyl)-[(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide [*3778-73-2*].

» Ifosfamide contains not less than 98.0 percent and not more than 102.0 percent of $C_7H_{15}Cl_2N_2O_2P$.

Caution—Great care should be taken in handling Ifosfamide, as it is a potent cytotoxic agent and suspected carcinogen.

Packaging and storage—Preserve in tight containers at a temperature not exceeding 25°.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩—*USP Endotoxin RS. USP Ifosfamide RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

pH ⟨791⟩: between 4.0 and 7.0 in a solution (1 in 10).

Water, *Method I* ⟨921⟩: not more than 0.3%.

Heavy metals, *Method I* ⟨231⟩: not more than 0.002%.

Ionic chloride—

Standard sodium chloride solution—Transfer about 118.7 mg of sodium chloride, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with water to volume, and mix. This solution contains 360 ppm of ionic chloride.

Procedure—Pipet 10 mL of *Standard sodium chloride solution* into a beaker, and add 90 mL of water and 10 mL of acetic acid. Titrate with 0.01 N silver nitrate VS (prepared fresh daily), determining the endpoint potentiometrically using silver and silver-silver chloride electrodes. Record the volume, V_1, of 0.01 N silver nitrate VS consumed. Transfer about 2.0 g of Ifosfamide, accurately weighed, into a beaker, and add 90 mL of water and 10 mL of acetic acid. Pipet 10 mL of *Standard sodium chloride solution* into the beaker, and stir, if necessary, until solution is complete. Titrate with 0.01 N silver nitrate VS as directed above, and record the volume, V_2, of 0.01 N silver nitrate VS consumed. Calculate the difference in volume, V, of 0.01 N silver nitrate VS consumed between the two determinations by subtracting V_1 from V_2: a difference of not more than 1.0 mL corresponding to not more than 0.018% of ionic chloride is found.

Chloroform-insoluble phosphorus—

Ammonium molybdate solution—[NOTE—Prepare fresh on the day of use.] Dissolve 25 g of ammonium molybdate in 300 mL of water (*Solution A*). Cautiously add 75 mL of sulfuric acid to 100 mL of water, cool to room temperature, and dilute with water to 200.0 mL (*Solution B*). Mix *Solution A* and *Solution B* to obtain *Ammonium molybdate solution*.

Hydroquinone solution—Dissolve 0.5 g of hydroquinone in 100 mL of water, and add one drop of concentrated sulfuric acid. [NOTE—When this solution darkens, discard it and prepare fresh.]

Sodium sulfite solution—Prepare a solution of sodium sulfite in water having a concentration of 200 mg per mL. [NOTE—Prepare fresh at the time of use.]

Phosphorus stock solution—Transfer 0.1824 g of monobasic potassium phosphate, accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Phosphorus intermediate solution—Transfer 10.0 mL of *Phosphorus stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Prepare this solution fresh on the day of use.

Phosphorus standard solution—Transfer 10.0 mL of *Phosphorus intermediate solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test preparation—Transfer 1 g of Ifosfamide, accurately weighed, to a 100-mL volumetric flask, dissolve in 50 mL of water, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a separatory funnel, and add 5 mL of water. Add 15 mL of chloroform, shake vigorously for 30 seconds, allow the layers to separate and drain, and discard the lower chloroform layer. Repeat this extraction four times, each time with 15 mL of chloroform, discarding the chloroform layer after each extraction. Transfer the aqueous portion to a conical flask, wash the separatory funnel with two 5-mL portions of water, and collect all the aqueous washings in the same flask. Add 3 mL of sulfuric acid, and heat under a hood until white fumes appear. Remove the flask from the heat, and with swirling, add 0.6 mL of hydrogen peroxide. Heat until white fumes reappear. If the solution is not colorless, repeat additions of hydrogen peroxide followed by heating until all color is gone. Cool to room temperature, add 25 mL of water, and cautiously add 10 mL of ammonium hydroxide solution. Cool to room temperature, add 2 drops of phenolphthalein TS, and then add hydrochloric acid dropwise until all pink color has disappeared. Transfer the contents of the flask to a 100-mL flask, dilute with water to volume, and mix.

Blank solution—To 3 mL of sulfuric acid in a second conical flask, adding 0.6 mL of hydrogen peroxide, proceed as directed for the *Test preparation*, beginning with "Heat until white fumes reappear."

Procedure—Transfer 15.0 mL each of the *Test preparation*, the *Blank solution*, and the *Phosphorus standard solution* to three separate 25-mL volumetric flasks. Add 2.5 mL of *Ammonium molybdate solution* to each of the flasks, swirl, and allow to stand for about 30 seconds. To each of the three flasks in order, rapidly add 2.5 mL each of *Hydroquinone solution* and *Sodium sulfite solution*. Dilute the contents of each flask with water to volume, mix, and allow the flasks to stand for 30 minutes. Concomitantly determine the absorbances of the solutions obtained from the *Test preparation* and the *Phosphorus standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 730 nm, with a suitable spectrophotometer, using the solution obtained from the *Blank solution* as the blank. Calculate the percentage of chloroform-insoluble phosphorus in the portion of Ifosfamide taken by the formula:

$$100(C / W)(A_U / A_S),$$

in which C is the concentration, in µg per mL, of phosphorus in the *Phosphorus standard solution*, W is the weight, in mg, of Ifosfamide taken, and A_U and A_S are the absorbances from the solutions obtained from the *Test preparation* and the *Phosphorus standard solution*, respectively: not more than 0.0415% is found.

Limit of 2-chloroethylamine hydrochloride—

Standard solution—Dissolve an accurately weighed quantity of 2-chloroethylamine hydrochloride in *N,N*-dimethylacetamide, and dilute quantitatively, and stepwise if necessary, with the same solvent to obtain a solution having a known concentration of about 0.025 mg per mL.

Test solution—Transfer about 100 mg of Ifosfamide, accurately weighed, to a flask, add 10.0 mL of *N,N*-dimethylacetamide, and shake until dissolved.

Chromatographic system—The gas chromatograph is equipped with a flame-ionization detector and contains a 2-mm ×

Levobunolol Hydrochloride Ophthalmic Solution

Levobunolol Hydrochloride Ophthalmic Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{25}NO_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Levobunolol Hydrochloride RS*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Antimicrobial effectiveness ⟨51⟩: meets the requirements.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 5.5 and 7.5.

Assay—

Mobile phase—Dissolve 990 mg of sodium 1-heptanesulfonate in 1000 mL of water, add 10 mL of glacial acetic acid and 1100 mL of methanol, mix, pass through a suitable filter having a 1-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Levobunolol Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.1 mg of levobunolol hydrochloride per mL.

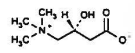
Chromatographic system (see *Chromatography* ⟨621⟩)—The chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the capacity factor, k', for levobunolol is between 1.2 and 1.4; the tailing factor for the analyte peak is not more than 2.6; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 30 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of $C_{17}H_{25}NO_3 \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

$$(L/D)(C)(r_U/r_S),$$

in which L is the labeled amount, in mg, of levobunolol hydrochloride in each mL of the Ophthalmic Solution; D is the concentration, in mg per mL, of levobunolol hydrochloride in the *Assay preparation*, based on the labeled quantity per mL and the extent of dilution; C is the concentration, in mg per mL, of USP Levobunolol Hydrochloride in the *Standard preparation;* and r_U and r_S are the peak area responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Levocarnitine



$C_7H_{15}NO_3$ 161.20

(*R*)-3-Carboxy-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide, inner salt.

(*R*)-(3-Carboxy-2-hydroxypropyl)trimethylammonium hydroxide, inner salt [*541-15-1*].

» Levocarnitine contains not less than 97.0 percent and not more than 103.0 percent of $C_7H_{15}NO_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Levocarnitine RS.*

Identification, *Infrared Absorption* ⟨197K⟩—The test specimen and the Reference Standard are dried previously in vacuum at 50° for 5 hours.

Specific rotation ⟨781S⟩: between −29° and −32°.

Test solution: 100 mg per mL, in water.

pH ⟨791⟩: between 5.5 and 9.5 in a solution (1 in 20).

Water content ⟨921⟩: not more than 4.0%.

Residue on ignition ⟨281⟩: not more than 0.5%.

Chloride ⟨221⟩—A 0.090-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.4%).

Limit of potassium—[NOTE—The *Standard solution* and the *Test solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard solution—Transfer 5.959 g of potassium chloride, previously dried at 105° for 2 hours and accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution contains 12.5 mg of potassium per mL. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with water to obtain a solution containing 31.25 µg of potassium per mL.

Test solutions—Transfer 62.5 mg of Levocarnitine to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a stock solution. To three separate 25-mL volumetric flasks add 0, 2.0, and 4.0 mL of the *Standard solution*. To each flask add 20.0 mL of the stock solution, dilute with water to volume, and mix. These solutions contain 0 (*Test solution A*), 2.5 (*Test solution B*), and 5.0 (*Test solution C*) µg per mL of potassium.

Procedure—Concomitantly determine the absorbances of the *Test solutions* at the potassium emission line at 766.7 nm with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-Scattering* ⟨851⟩) equipped with an air–acetylene flame, using water as the blank. Plot the absorbances of the *Test solutions* versus their contents of potassium, in µg per mL, draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg, of potassium in each mL of *Test solution A*. Calculate the percentage of potassium in the portion of Levocarnitine taken by multiplying the concentration, in µg per mL, of potassium found in *Test solution A* by 0.2: not more than 0.2% is found.

Limit of sodium—[NOTE—The *Standard solution* and the *Test solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

Standard solution—Transfer 6.355 g of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10.0 mg of sodium per mL. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with water to obtain a solution containing 250 µg of sodium per mL.

Assay preparation—To about 12 mg of Lincomycin Hydrochloride, accurately weighed, add 10.0 mL of *Mobile phase.* Shake by mechanical means for 5 minutes, and sonicate if necessary to effect solution.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7 and is maintained at a temperature of 46°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the tailing factor for the main lincomycin peak is not more than 1.3; the column efficiency determined from the main lincomycin peak is not less than 4000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. The relative retention times are about 0.5 for lincomycin B and 1.0 for lincomycin. Calculate the quantity, in µg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mg of the Lincomycin Hydrochloride taken by the formula:

$$10(CP/W)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Lincomycin Hydrochloride RS in the *Standard preparation; P* is the designated potency, in µg of lincomycin per mg, of USP Lincomycin Hydrochloride RS; W is the weight, in mg, of the portion of Lincomycin Hydrochloride taken to prepare the *Assay preparation;* and r_U and r_S are the lincomycin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lincomycin Hydrochloride Capsules

» Lincomycin Hydrochloride Capsules contain an amount of $C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Lincomycin Hydrochloride RS.*

Dissolution ⟨711⟩—
Medium: water; 500 mL.
Apparatus 1: 100 rpm.
Time: 45 minutes.

Procedure—Filter a portion of about 20 mL of the solution under test. Transfer about 5 mL of the eluant into a small test tube, and add 250 µL of 0.01 M sodium sulfate internal standard solution. Evaporate until dry using a vacuum centrifuge. Add 10.0 µL of water to the precipitate and place on a vortex mixer until all solid material is dissolved. Transfer this solution to a capillary tube, place it in a Raman spectrometer, and obtain the Raman spectrum using suitable instrumental conditions (see *Spectrophotometry and Light-Scattering* ⟨851⟩). Integrate the Raman intensity, applying baseline corrections, between 660 cm^{-1} and 720 cm^{-1}. Divide this result by the integrated intensity between 966 cm^{-1} and 994 cm^{-1}. Determine the amount of $C_{18}H_{34}N_2O_6S$ dissolved in comparison with an aqueous Standard solution having a known concentration of USP Lincomycin Hydrochloride RS.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{18}H_{34}N_2O_6S$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water, *Method I* ⟨921⟩: not more than 7.0%.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*

Assay preparation—Remove, as completely as possible, the contents of not less than 10 Capsules, taking care to prevent capsule shell fragments from being combined with the capsule contents and to remove any shell fragments from the contents. Weigh and mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of lincomycin, to a suitable container. Add 50.0 mL of *Mobile phase*, and shake by mechanical means for 5 minutes. Use the solution thus obtained as the *Assay preparation.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride.* Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in the portion of Capsule contents taken by the formula:

$$(CP/20)(r_U/r_S),$$

in which the terms are as defined therein.

Lincomycin Injection

» Lincomycin Injection contains an amount of Lincomycin Hydrochloride in Water for Injection equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$). It contains benzyl alcohol as a preservative.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards ⟨11⟩—*USP Endotoxin RS. USP Lincomycin Hydrochloride RS.*

Bacterial endotoxins ⟨85⟩—It contains not more than 0.5 USP Endotoxin Unit per mg of lincomycin.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 3.0 and 5.5.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—
Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Lincomycin Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 600 mg of lincomycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Lincomycin Hydrochloride.* Calculate the quantity, in mg, of lincomycin ($C_{18}H_{34}N_2O_6S$) in each mL of the Injection taken by the formula:

$$0.625(CP/V)(r_U/r_S),$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Lincomycin Hydrochloride Soluble Powder

» Lincomycin Hydrochloride Soluble Powder contains an amount of Lincomycin Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lincomycin ($C_{18}H_{34}N_2O_6S$).

relative response factor, F_{mi}, of equal weights of toluene and methyl iodide taken by the formula:

$$Q_{smi} / A_{smi},$$

in which Q_{mi} is the quantity ratio of methyl iodide to toluene in the *Standard preparation*, and A_{smi} is the peak area ratio of the methyl iodide to toluene obtained from the *Standard preparation*.

Procedure—Inject about 2 µL of the upper layer of the *Assay preparation* into the gas chromatograph, and record the chromatogram. Calculate the percentage of methoxy in the Methylcellulose taken by the formula:

$$2(31 / 142)F_{mi}A_{umi}(W_t / W_u),$$

in which 31/142 is the ratio of the formula weights of methoxy and methyl iodide; F_{mi} is defined under *Calibration*, A_{umi} is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay preparation*; W_t is the weight, in g, of toluene in the *Internal standard solution*; and W_u is the weight, in g, of Methylcellulose taken for the *Assay*.

Methylcellulose Ophthalmic Solution

» Methylcellulose Ophthalmic Solution is a sterile solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose. It may contain suitable antimicrobial, buffering, and stabilizing agents.

Packaging and storage—Preserve in tight containers.

Identification—It responds to *Identification* tests *B* and *C* under *Methylcellulose*.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 6.0 and 7.8.

Assay—To boiling flask *A*, as described under *Methoxy Determination* ⟨431⟩, pipet a quantity of Ophthalmic Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* ⟨431⟩. Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Oral Solution

» Methylcellulose Oral Solution is a flavored solution of Methylcellulose. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat. Avoid freezing.

Identification—It responds to *Identification* tests *B* and *C* under *Methylcellulose*.

Microbial limits ⟨61⟩—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

Alcohol content, *Method II* ⟨611⟩: between 3.5% and 6.5% of C_2H_5OH.

Assay—To boiling flask *A*, as described under *Methoxy Determination* ⟨431⟩, transfer an accurately measured volume of Oral Solution, equivalent to 50 mg of methylcellulose. Evaporate on a steam bath to dryness, cool the flask in an ice bath, add the specified amount of hydriodic acid, and proceed as directed under *Methoxy Determination* ⟨431⟩. Each mL of 0.1 N sodium thiosulfate is equivalent to 1.753 mg of methylcellulose.

Methylcellulose Tablets

» Methylcellulose Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of methylcellulose.

Packaging and storage—Preserve in well-closed containers.

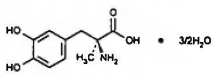
Identification—Add the residue obtained in the *Assay* to 50 mL of water: the solution responds to the *Identification* tests under *Methylcellulose*.

Disintegration ⟨701⟩: 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of methylcellulose, and transfer to a tared, fine fritted-glass, low-form, 30-mL crucible having a fitted crucible lid. Add 20 mL of alcohol, and macerate the solid for about 5 minutes, mixing intermittently with a glass stirring rod. Repeat the extraction with ten consecutive 10-mL portions of alcohol. Test for completeness of extraction by evaporating the last alcohol extract on a steam bath to dryness, taking up the residue in about 1 mL of water, and adding this to 5 mL of hot alkaline cupric tartrate TS (no red precipitate of cuprous oxide is formed within 5 minutes). If a precipitate is formed, continue with the alcohol extractions until the test is negative. Wash the completely extracted residue with a 10-mL portion of ether, using suction to draw off the liquid. Dry the residue in the crucible in a drying oven at 105° to constant weight. Weigh the crucible with the crucible lid in place. The weight of residue is the weight of methylcellulose present in the portion of powdered Tablets taken.

Methyldopa



$C_{10}H_{13}NO_4 \cdot 1½H_2O$ 238.24

L-Tyrosine, 3-hydroxy-α-methyl-, sesquihydrate.

L-3-(3,4-Dihydroxyphenyl)-2-methylalanine sesquihydrate [*41372-08-1*].

Anhydrous 211.22 [*555-30-6*].

» Methyldopa contains not less than 98.0 percent and not more than 101.0 percent of $C_{10}H_{13}NO_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Methyldopa RS*. *USP 3-O-Methylmethyldopa RS*.

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 40 µg per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 280 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

C: To 10 mg add 0.15 mL of a solution of ninhydrin in sulfuric acid (1 in 250): a dark purple color is produced within 5 to 10 minutes. Add 0.15 mL of water: the color changes to pale brownish yellow.

Specific rotation ⟨781S⟩: between –25° and –28°.

Test solution: 44 mg per mL, in a solvent that is a solution of aluminum chloride in water (2 in 3) which previously has been treated with activated charcoal, filtered, and adjusted with 0.25 N sodium hydroxide to a pH of 1.5.

Naphazoline Hydrochloride Nasal Solution

» Naphazoline Hydrochloride Nasal Solution is a solution of Naphazoline Hydrochloride in water adjusted to a suitable pH and tonicity. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Naphazoline Hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Naphazoline Hydrochloride RS.*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay.*

Assay—

Mobile phase—Dissolve 1.1 g of sodium 1-heptanesulfonate in about 400 mL of water. Add 250 mL of acetonitrile and 10 mL of glacial acetic acid, dilute with water to 1000 mL, and mix. Sonicate for 10 minutes, filter, and degas to obtain a solution having a pH of about 3.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Naphazoline Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 250 µg per mL.

Assay preparation—Pipet a volume of Nasal Solution, equivalent to about 25 mg of naphazoline hydrochloride, into a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the tailing factor for the naphazoline hydrochloride peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{14}N_2 \cdot HCl$ in each mL of the Nasal Solution taken by the formula:

$$0.1(C/V)(r_U/r_S),$$

in which C is the concentration, in µg per mL, of USP Naphazoline Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Nasal Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naphazoline Hydrochloride Ophthalmic Solution

» Naphazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride in water adjusted to a suitable tonicity. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of Naphazoline Hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$). It contains a suitable preservative.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Naphazoline Hydrochloride RS.*

Identification—Place in a separator a volume of Ophthalmic Solution, equivalent to about 25 mg of naphazoline hydrochloride, add 5 mL of 1 N sodium hydroxide, saturate with sodium chloride, and extract with two 25-mL portions of ether. Wash the ether solution with 5 mL of water, pass the ether through a small paper, evaporate the filtrate to about 5 mL, transfer the residual solution to a 10- to 15-mL beaker, allow to evaporate spontaneously, and dry the residue at 80° for 1 hour: the naphazoline so obtained melts between 115° and 120° when determined as directed for *Class Ia Melting Range* or *Temperature* ⟨741⟩.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.5 and 7.0.

Assay—

Phosphate buffer—Transfer 3 g of monobasic potassium phosphate to a 1-liter volumetric flask, dissolve in 1000 mL of water and 2 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0 and mix.

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer* and acetonitrile (80 : 20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Naphazoline Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 5.0 mg of naphazoline hydrochloride, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 285-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 40°. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{14}N_2 \cdot HCl$ in the portion of Ophthalmic Solution taken by the formula:

$$100C(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Naphazoline Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution

» Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution is a sterile, buffered solution of Naphazoline Hydrochloride and Pheniramine Maleate in water adjusted to a suitable tonicity. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) and pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$). It contains a suitable preservative.

Packaging and storage—Preserve in tight containers, and store at temperature between 20° and 25°, protected from light.

USP Reference standards ⟨11⟩—*USP Naphazoline Hydrochloride RS. USP Pheniramine Maleate RS.*

Identification—

A: Proceed as directed in the following thin-layer chromatographic procedure.

Naphazoline hydrochloride standard solution—Dissolve a quantity of USP Naphazoline Hydrochloride RS in water

Pheniramine maleate standard solution—Dissolve a quantity of USP Pheniramine Maleate RS in water to obtain a solution containing about 6.0 mg per mL.

Test solution—Dilute, if necessary, a volume of Ophthalmic Solution with water to obtain a solution containing about 0.25 mg of naphazoline hydrochloride per mL and 3 mg of pheniramine maleate per mL.

Procedure—Separately apply 5 µL of *Naphazoline hydrochloride standard solution*, 10 µL of *Pheniramine maleate standard solution*, and 30 µL of the *Test solution* to a 20-cm × 20-cm thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of silica gel. Allow the spots to dry, then place the plate in a saturated chromatographic chamber, and develop in a solvent system consisting of methanol, water, and acetic acid (8 : 1 : 1) until the solvent front has moved to about 1.5 cm from the top of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray with ninhydrin TS, and place in an oven at 105° to visualize the spots. Both the naphazoline and pheniramine spots are purplish grey in color. The R_F values of the spots obtained from the *Test solution* correspond to those obtained from the *Naphazoline hydrochloride standard solution* and the *Pheniramine maleate standard solution*.

B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those of the *Standard preparation,* as obtained in the *Assay.*

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 5.7 and 6.3.

Assay—

Buffer solution—Dissolve 14.2 g of anhydrous dibasic sodium phosphate and 20 mL of triethylamine in 1900 mL of water, adjust with phosphoric acid to a pH of 5.6 ± 0.1, dilute with water to make 2000 mL of solution, and mix.

Mobile phase—Prepared a filtered and degassed mixture of *Buffer* and acetonitrile (80 : 20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Naphazoline hydrochloride stock standard solution—Dissolve an accurately weighed quantity of USP Naphazoline Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.75 mg per mL.

Pheniramine maleate stock standard solution—Dissolve an accurately weighed quantity of USP Pheniramine Maleate RS in *Mobile phase* to obtain a known concentration of about 3.00 mg per mL.

Standard preparation—Transfer 1.0 mL of *Naphazoline hydrochloride stock standard solution* and 3.0 mL of *Pheniramine maleate stock standard solution* to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having known concentrations of naphazoline hydrochloride and pheniramine maleate of 0.03 and 0.36 mg per mL, respectively.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 0.75 mg of naphazoline hydrochloride and 9.0 mg of pheniramine maleate, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

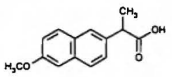
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the naphazoline peak and the pheniramine peak is not less than 2; the column efficiency, determined from the naphazoline and pheniramine peaks, is not less than 750 theoretical plates; the tailing factor is not greater than 2.5 for pheniramine; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the peaks. Calculate the quantity, in mg, of naphazoline hydrochloride ($C_{14}H_{14}N_2 \cdot HCl$) in each mL of the Ophthalmic Solution taken by the formula:

$$25(C/V)(r_U/r_S),$$

in which C is the concentration in mg per mL of USP Naphazoline Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Ophthalmic solution taken; and r_U and r_S are the naphazoline peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively. Calculate the quantity, in mg, of pheniramine maleate ($C_{16}H_{20}N_2 \cdot C_4H_4O_4$) in each mL of the Ophthalmic Solution taken by the same formula, changing the terms to refer to pheniramine maleate.

Naproxen



$C_{14}H_{14}O_3$ 230.26

2-Naphthaleneacetic acid, 6-methoxy-α-methyl-, (*S*)-.

(+)-(*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid [*22204-53-1*].

» Naproxen contains not less than 98.5 percent and not more than 101.5 percent of $C_{14}H_{14}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Naproxen RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 25 µg per mL.

Medium: methanol.

Absorptivities at 271 nm, calculated on the dried basis, do not differ by more than 3%.

Specific rotation ⟨781S⟩: between +63.0° and +68.5°.

Test solution: 10 mg per mL, in chloroform.

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Chromatographic purity—Dissolve 100 mg of Naproxen in methanol, and dilute with methanol to 5.0 mL to obtain the *Test solution.* Dissolve a suitable quantity of USP Naproxen RS in methanol to obtain a *Standard solution* having a known concentration of about 20 mg per mL. Dilute a portion of this solution quantitatively and stepwise with methanol to obtain three *Comparison solutions* having concentrations of 20, 60, and 100 µg per mL (0.1%, 0.3%, and 0.5% of the *Standard solution*), respectively. Apply separate 10-µL portions of the five solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of toluene, tetrahydrofuran, and glacial acetic acid (30 : 3 : 1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, air-dry, and view under short-wavelength UV light: the R_F value of the principal spot in the chromatogram of the *Test solution* corresponds to that of the *Standard solution,* and any other spot obtained from the *Test solution* does not exceed, in size or intensity, the principal spot obtained from the 100-µg-per-mL *Comparison solution* (0.5%), and the sum of the intensities of any secondary spots, similarly compared, does not exceed 2.0%.

Organic volatile impurities, *Method V* ⟨467⟩: meets the requirements.

Solvent—Use dimethyl sulfoxide.

Assay—Dissolve about 500 mg of Naproxen, accurately weighed, in a mixture of 75 mL of methanol and 25 mL of water that has been previously neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide. Dissolve by gentle warming, if necessary, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 23.03 mg of $C_{14}H_{14}O_3$.

mg of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Cream taken by the formula:

$$0.1C(r_U/r_S).$$

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment

» Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$).

NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Ointment is prescribed without reference to the quantity of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg of neomycin and 0.5 mg of dexamethasone phosphate per g shall be dispensed.

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes.

USP Reference standards ⟨11⟩—*USP Dexamethasone RS. USP Dexamethasone Phosphate RS. USP Neomycin Sulfate RS.*

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: The *Assay preparation*, prepared as directed in the *Assay for dexamethasone phosphate*, meets the requirements for the *Identification* test under *Dexamethasone Sodium Phosphate Cream.*

Sterility ⟨71⟩: meets the requirements.

Minimum fill ⟨755⟩: meets the requirements.

Water, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7 : 3) being used in place of methanol in the titration vessel.

Metal particles—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* ⟨751⟩.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Ophthalmic Ointment shaken in a separator with about 50 mL of water, and extracted with four 20-mL portions of *Buffer No. 3*. Combine the aqueous extracts, and dilute with *Buffer No. 3* to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer No. 3* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for dexamethasone phosphate—

Alcohol–aqueous phosphate buffer, *0.05 M Phosphate buffer*, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Dexamethasone Sodium Phosphate Cream.*

Assay preparation—Using an accurately weighed portion of Ophthalmic Ointment, prepare as directed in the *Assay* under *Dexamethasone Sodium Phosphate Cream.*

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Dexamethasone Sodium Phosphate Cream.* Calculate the quantity, in mg, of dexamethasone phosphate ($C_{22}H_{30}FO_8P$) in the portion of Ophthalmic Ointment taken by the formula:

$$0.1C(r_U/r_S).$$

Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution

» Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is a sterile, aqueous solution of Neomycin Sulfate and Dexamethasone Sodium Phosphate. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of dexamethasone phosphate ($C_{22}H_{30}FO_8P$). It may contain one or more suitable buffers, dispersants, and preservatives.

NOTE—Where Neomycin Sulfate and Dexamethasone Sodium Phosphate Ophthalmic Solution is prescribed, without reference to the amount of neomycin or dexamethasone phosphate contained therein, a product containing 3.5 mg of neomycin and 1.0 mg of dexamethasone phosphate per mL shall be dispensed.

Packaging and storage—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—*USP Dexamethasone RS. USP Dexamethasone Phosphate RS. USP Neomycin Sulfate RS.*

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: The *Assay preparation*, prepared as directed in the *Assay for dexamethasone phosphate*, meets the requirements for the *Identification* test under *Dexamethasone Sodium Phosphate Cream.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 6.0 and 8.0.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of neomycin per mL).

Assay for dexamethasone phosphate—

0.002 M Phosphate buffer—Dissolve 0.57 g of dibasic sodium phosphate in water to obtain 2000 mL of solution.

0.10 M Phosphate buffer—Dissolve 13.80 g of monobasic sodium phosphate in water to obtain 1000 mL of solution.

Mobile phase—Prepare a suitable filtered mixture of *0.10 M phosphate buffer* and acetonitrile (690 : 310). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone Phosphate RS in *0.002 M Phosphate buffer* to obtain a solution having a known concentration of about 125 µg per mL. Transfer 20.0 mL of this solution to a 100-mL volumetric flask, dilute with *0.002 M Phosphate buffer* to volume, mix, and pass through a suitable filter of 1 µm or finer porosity. This solution contains about 25 µg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 2.5 mg of dexamethasone phosphate, to a 100-mL volumetric flask, slowly dilute with *0.002 M Phosphate buffer* to volume, mix, and pass through a suitable filter of 1 µm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.3 mL per minute. Chromatograph the *Standard preparation*, and measure the peak responses as directed under *Procedure:* the column efficiency is not less than 2000 theoretical plates, the capacity factor, k', for the dexamethasone phosphate peak is not less than 1.05, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the

major peaks. Calculate the quantity, in mg, of dexamethasone phosphate ($C_{22}H_{30}FO_8P$), in each mL of the Ophthalmic Solution taken by the formula:

$$0.1(C/V)(r_U/r_S),$$

in which C is the concentration, in µg per mL, of USP Dexamethasone Phosphate RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the dexamethasone phosphate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Fluocinolone Acetonide Cream

» Neomycin Sulfate and Fluocinolone Acetonide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers.

USP Reference standards ⟨11⟩—*USP Fluocinolone Acetonide RS. USP Neomycin Sulfate RS.*

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: It meets the requirements for the *Identification* test under *Fluocinolone Acetonide Cream.*

Minimum fill ⟨755⟩: meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Cream.*

Assay for fluocinolone acetonide—Proceed with Cream as directed in the *Assay* under *Fluocinolone Acetonide Cream.*

Neomycin Sulfate and Fluorometholone Ointment

» Neomycin Sulfate and Fluorometholone Ointment contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of fluorometholone ($C_{22}H_{29}FO_4$).

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards ⟨11⟩—*USP Fluorometholone RS. USP Neomycin Sulfate RS.*

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: The ratios of the retention time of the main peak to that of the internal standard peak obtained from the *Standard preparation* and the *Assay preparation* as directed in the *Assay for fluorometholone* do not differ by more than 2.0%.

Minimum fill ⟨755⟩: meets the requirements.

Water, *Method I* ⟨921⟩: not more than 1.0%, 20 mL of a mixture of toluene and methanol (7 : 3) being used in place of methanol in the titration vessel.

Assay for neomycin—Proceed with Ointment as directed in the *Assay* under *Neomycin Sulfate Ointment.*

Assay for fluorometholone—

Internal standard solution, Mobile solvent, and *Standard preparation*—Prepare as directed in the *Assay* under *Fluorometholone Cream.*

Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 1 mg of fluorometholone, to a suitable container, add 20.0 mL of *Internal standard solution,* and mix.

Procedure—Treat 20.0 mL each of the *Standard preparation* and the *Assay preparation* in the following manner. To each add 10.0 mL of hexane, shake for about 15 minutes, then allow the layers to separate, and centrifuge, if necessary. Using the lower (acetonitrile) layer, proceed as directed for *Procedure* in the *Assay* under *Fluorometholone Cream,* beginning with "Using a suitable microsyringe." Calculate the quantity, in mg, of $C_{22}H_{29}FO_4$ in the portion of Ointment taken by the formula:

$$20C(R_U/R_S),$$

in which the terms are as defined therein.

Neomycin Sulfate and Flurandrenolide Cream

» Neomycin Sulfate and Flurandrenolide Cream contains the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in collapsible tubes or in tight containers, protected from light.

USP Reference standards ⟨11⟩—*USP Flurandrenolide RS. USP Neomycin Sulfate RS.*

Identification—

A: It meets the requirements for neomycin under *Thin-Layer Chromatographic Identification Test* ⟨201BNP⟩.

B: It meets the requirements for the *Identification* test under *Flurandrenolide Cream.*

Minimum fill ⟨755⟩: meets the requirements.

Assay for neomycin—Proceed with Cream as directed in the *Assay* under *Neomycin Sulfate Ointment.*

Assay for flurandrenolide—Proceed with Cream as directed in the *Assay* under *Flurandrenolide Cream.* Calculate the quantity, in mg, of $C_{24}H_{33}FO_6$ in the portion of Cream taken by the formula:

$$10C(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Flurandrenolide RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin Sulfate and Flurandrenolide Lotion

» Neomycin Sulfate and Flurandrenolide Lotion contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of flurandrenolide ($C_{24}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers, protected from light.

Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately weighed portion of Cream shaken with about 50 mL of ether in a separator, and extracted with four 25-mL portions of *Buffer No. 6*. Combine the aqueous extracts, and dilute with *Buffer No. 6* to an appropriate volume to obtain a stock solution. Dilute this stock solution quantitatively and stepwise with *Buffer No. 6* to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer No. 6*, to obtain the same concentration of neomycin present in the *Test Dilution*.

Neomycin and Polymyxin B Sulfates Solution for Irrigation

» Neomycin and Polymyxin B Sulfates Solution for Irrigation is a sterile, aqueous solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It may contain a suitable preservative.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is to be diluted for use in a urinary bladder irrigation and is not intended for injection.

USP Reference standards ⟨11⟩—*USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 4.5 and 6.0.

Assay for neomycin and Assay for polymyxin B—Proceed with Neomycin and Polymyxin B Sulfates Solution for Irrigation as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

Neomycin and Polymyxin B Sulfates Ophthalmic Ointment

» Neomycin and Polymyxin B Sulfates Ophthalmic Ointment is a sterile ointment containing Neomycin Sulfate and Polymyxin B Sulfate. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B.

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes.

USP Reference standards ⟨11⟩—*USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Minimum fill ⟨755⟩: meets the requirements.

Water, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7 : 3) being used in place of methanol in the titration vessel.

Metal particles—It meets the requirements of the test for *Metal Particles in Ophthalmic Ointments* ⟨751⟩.

Assay for neomycin and Assay for polymyxin B—Proceed with Ophthalmic Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Neomycin and Polymyxin B Sulfates Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates Ophthalmic Solution contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It may contain one or more suitable buffers, dispersants, irrigants, and preservatives.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩—*USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 5.0 and 7.0.

Assay for neomycin and Assay for polymyxin B—Proceed with Ophthalmic Solution as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution.*

Neomycin and Polymyxin B Sulfates and Bacitracin Ointment

» Neomycin and Polymyxin B Sulfates and Bacitracin Ointment contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and bacitracin. It may contain a suitable local anesthetic.

Packaging and storage—Preserve in tight, light-resistant containers, preferably at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Bacitracin Zinc RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Minimum fill ⟨755⟩: meets the requirements.

Water, *Method I* ⟨921⟩: not more than 0.5%, 20 mL of a mixture of toluene and methanol (7 : 3) being used in place of methanol in the titration vessel.

Assay for neomycin and Assay for polymyxin B—Proceed with Ointment as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Assay for bacitracin—Proceed with Ointment as directed in the *Assay* under *Bacitracin Ointment.*

4.6-mm × 25-cm column that contains 5- to 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed under *Procedure:* the column efficiency is not less than 4000 theoretical plates, and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{22}H_{39}FO_5$ in the portion of Ophthalmic Ointment taken by the formula:

$$50C(r_U / r_S),$$

in which C is the concentration, in µg per mL, of USP Dexamethasone RS in the *Standard preparation;* and r_U and r_S are the peak responses of the *Assay preparation* and the *Standard preparation*, respectively.

Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Suspension contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone. It may contain one or more suitable buffers, stabilizers, preservatives, and suspending agents.

Packaging and storage—Preserve in tight, light-resistant containers in a cool place or at controlled room temperature. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—*USP Dexamethasone RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Identification—Transfer a quantity of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a suitable test tube, add 5 mL of chloroform, mix, and centrifuge. Apply 25 µL of the lower chloroform layer and 25 µL of a Standard solution of USP Dexamethasone RS in chloroform containing 500 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and diethylamine (2 : 1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 3.5 and 6.0.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer No. 3* to yield *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer No. 6* to yield *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer No. 6*, to obtain the same concentration of neomycin as is present in the *Test Dilution*.

Assay for dexamethasone—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment.*

Standard preparation—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Mobile phase* to obtain a solution having a known concentration of about 0.12 mg per mL.

Assay preparation—Dilute an accurately measured volume of freshly mixed Ophthalmic Suspension quantitatively with *Mobile phase* to obtain a solution containing about 0.12 mg of dexamethasone per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay for dexamethasone* under *Neomycin and Polymyxin B Sulfates and Dexamethasone Ophthalmic Ointment.* Calculate the quantity, in mg per mL, of $C_{22}H_{39}FO_5$ in the Ophthalmic Suspension taken by the formula:

$$(CL / D)(r_U / r_S),$$

in which L is the labeled quantity, in mg per mL, of dexamethasone in the Ophthalmic Suspension, D is the concentration, in mg per mL, of dexamethasone in the *Assay preparation* based on the labeled quantity in the Ophthalmic Suspension and the extent of dilution, and the other terms are as defined therein.

Neomycin and Polymyxin B Sulfates and Gramicidin Cream

» Neomycin and Polymyxin B Sulfates and Gramicidin Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in collapsible tubes or in well-closed containers.

USP Reference standards ⟨11⟩—*USP Gramicidin RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Minimum fill ⟨755⟩: meets the requirements.

Assay for neomycin and Assay for polymyxin B—Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment.*

Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution

» Neomycin and Polymyxin B Sulfates and Gramicidin Ophthalmic Solution is a sterile, isotonic aqueous solution of Neomycin Sulfate, Polymyxin B Sulfate, and Gramicidin. It contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—*USP Gramicidin RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 4.7 and 6.0.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. Add to each test dilution of the Standard a quantity of USP Neomycin Sulfate RS, dissolved in *Buffer No. 6*, to obtain the same concentration of neomycin as is present in the *Test Dilution.*

Assay for gramicidin—Proceed as directed for gramicidin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Solution diluted quantitatively and stepwise with alcohol to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream

» Neomycin and Polymyxin B Sulfates, Gramicidin, and Hydrocortisone Acetate Cream contains the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin, polymyxin B, and gramicidin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate ($C_{23}H_{32}O_6$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP Gramicidin RS. USP Hydrocortisone Acetate RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Minimum fill ⟨755⟩: meets the requirements.

Assay for neomycin and Assay for polymyxin B—Proceed with Cream as directed in the *Assay for neomycin* and in the *Assay for polymyxin B* under *Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment.*

Assay for gramicidin—Proceed with Cream as directed in the *Assay for gramicidin* under *Neomycin Sulfate and Gramicidin Ointment.*

Assay for hydrocortisone acetate—Proceed with Cream as directed in the *Assay* under *Hydrocortisone Acetate Lotion.*

Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Otic Solution is a sterile solution containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone. It may contain one or more suitable buffers, dispersants, and solvents.

Packaging and storage—Preserve in tight, light-resistant containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—*USP Hydrocortisone RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 2.0 and 4.5.

Assay for neomycin—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (1.0 µg of neomycin per mL).

Assay for polymyxin B—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Otic Solution diluted quantitatively and stepwise with *Buffer No. 6* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard (10 Polymyxin B Units per mL). Add to each test dilution of the Standard a quantity of Neomycin Standard, dissolved in *Buffer No. 6*, to obtain the same concentration of neomycin present in the *Test Dilution.*

Assay for hydrocortisone—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.*

Assay preparation—Transfer 3.0 mL of Otic Solution to a 200-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Neomycin and Polymyxin B Sulfates, Bacitracin Zinc and Hydrocortisone Ophthalmic Ointment.* Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in each mL of the Otic Solution taken by the formula:

$$(66.67C)(r_U / r_S),$$

in which C is the concentration, in mg per mL, of USP Hydrocortisone RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension

» Neomycin and Polymyxin B Sulfates and Hydrocortisone Ophthalmic Suspension is a sterile, aqueous suspension containing the equivalent of not less than 90.0 percent and not more than 130.0 percent of the labeled amounts of neomycin and of polymyxin B. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone.

Packaging and storage—Preserve in tight containers. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

USP Reference standards ⟨11⟩—*USP Hydrocortisone RS. USP Neomycin Sulfate RS. USP Polymyxin B Sulfate RS.*

Thin-layer chromatographic identification test ⟨201BNP⟩: meets the requirements.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 4.1 and 7.0.

Assay for neomycin—Proceed as directed for neomycin under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, diluted quantitatively and stepwise with *Buffer No. 3* to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

Assay for polymyxin B—Proceed as directed for polymyxin B under *Antibiotics—Microbial Assays* ⟨81⟩, using an accurately m

Norfloxacin Ophthalmic Solution

» Norfloxacin Ophthalmic Solution is a sterile, aqueous solution of Norfloxacin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norfloxacin ($C_{16}H_{18}FN_3O_3$).

Packaging and storage—Preserve in tight, light-resistant containers, stored at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Norfloxacin RS.*

Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—

Solution: about 0.06 mg of norfloxacin per mL.

Diluent: 0.1 N hydrochloric acid.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.0 and 5.4.

Assay—

Dilute phosphoric acid solution—Prepare a solution of phosphoric acid in water (1 in 1000).

Mobile phase—Prepare a filtered and degassed mixture of *Dilute phosphoric acid solution* and acetonitrile (850 : 150). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Prepare a solution of USP Norfloxacin RS in *Dilute phosphoric acid solution* having a known concentration of about 0.06 mg per mL.

Resolution solution—Prepare a solution of USP Norfloxacin RS and pipemidic acid in *Dilute phosphoric acid solution* having known concentrations of about 0.06 mg of each per mL.

Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively and stepwise with *Dilute phosphoric acid solution* to obtain a solution having a concentration of about 0.06 mg of norfloxacin per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The column temperature is maintained at 50°. The flow rate is about 0.5 mL per minute. Precondition the column for about 8 hours with 0.01 M monobasic sodium phosphate buffer adjusted with phosphoric acid to a pH of 4.0. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for pipemidic acid and 1.0 for norfloxacin; and the resolution, *R*, between the pipemidic acid peak and the norfloxacin peak is not less than 1.2. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor for the norfloxacin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of norfloxacin ($C_{16}H_{18}FN_3O_3$) in each mL of the Ophthalmic Solution taken by the formula:

$$(L/D)(C)(r_U/r_S),$$

in which L is the labeled quantity, in mg per mL, of norfloxacin in the Ophthalmic Solution; D is the concentration, in mg per mL, of norfloxacin in the *Assay preparation*, based on the labeled quantity of norfloxacin in each mL of the Ophthalmic Solution and the extent of dilution; C is the concentration, in mg per mL, of USP Norfloxacin RS in the *Standard preparation;* and r_U and r_S are the norfloxacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norfloxacin Tablets

» Norfloxacin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Norfloxacin ($C_{16}H_{18}FN_3O_3$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP Norfloxacin RS.*

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* obtained as directed in the *Assay.*

B: Shake a quantity of finely powdered Tablets, equivalent to about 75 mg of norfloxacin, with 50 mL of a mixture of acidic methanol (prepared by mixing 1000 mL of methanol and 9 mL of hydrochloric acid) and methylene chloride (1 : 1). Centrifuge a portion of the suspension thus obtained, and use the clear supernatant as the test solution. Apply 50 µL each of the test solution and a standard solution of USP Norfloxacin RS in the same solvent containing 1.5 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a suitable chromatographic chamber that contains and has been equilibrated with a developing system consisting of a mixture of chloroform, methanol, toluene, diethylamine, and water (40 : 40 : 20 : 14 : 8), and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—

pH 4.0 buffer—To 900 mL of water in a 1000-mL volumetric flask add 2.86 mL of glacial acetic acid and 1.0 mL of a 50% (w/w) solution of sodium hydroxide, dilute with water to volume, and mix. If necessary, adjust with glacial acetic acid or the sodium hydroxide solution to a pH of 4.0.

Medium: pH 4.0 buffer; 750 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{16}H_{18}FN_3O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 278 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Norfloxacin RS in the same medium.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{16}H_{18}FN_3O_3$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of phosphoric acid solution (1 in 1000) and acetonitrile (850 : 150). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Norfloxacin RS quantitatively in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder equivalent to about 100 mg of norfloxacin, to a 200-mL volumetric flask. Add 80 mL of *Mobile phase*, sonicate for 10 minutes, dilute with phosphoric acid solution (1 in 1000) to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, mix, and filter through a filter having porosity of 1 µm or less.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 275-nm detector and a 3.9-mm × 30-cm column that contains packing L1, and is operated at 40 ± 1.0°.

Precondition the column with degassed 0.01 M monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.0, flowing at a rate of 0.5 mL per minute for 8 hours. For the assay, use a *Mobile phase* flow rate of about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed

and r_i is the total area of the peaks, except for the solvent peak: not more than 0.2% is found. Calculate the percentage of each other impurity with an area greater than that of the ofloxacin peak in the chromatogram of the *System suitability standard solution* obtained under *Chromatographic system,* by the formula:

$$100(r_i / r_t),$$

in which r_i is the peak area for an individual impurity; and r_t is the total area of the peaks in the chromatogram obtained from the *Test solution,* except for the solvent peak: not more than 0.3% of any individual impurity is found; and the sum of all impurities found is not more than 0.5%.

Limit of methanol and ethanol—

Internal standard solution—Prepare a solution in sodium hydroxide solution (1 in 100) containing 0.7 µL of *n*-propyl alcohol per mL. Transfer 2.0 mL of this solution to a 250-mL volumetric flask, dilute with the same sodium hydroxide solution (1 in 100) to volume, and mix.

Standard solution—Prepare a solution in *Internal standard solution* containing 10.0 µg each of methanol and dehydrated alcohol per mL. Transfer 2.0 mL of this solution to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 minutes, and shake for 6 minutes.

Test solution—Transfer 40 mg of Ofloxacin, accurately weighed, to a vial fitted with a septum and a crimp cap, add 2.0 mL of *Internal standard solution,* and seal the vial. Heat the sealed vial at 90° for 2 minutes, and shake for 6 minutes.

Blank—Transfer 2.0 mL of the *Internal standard solution* to a vial fitted with a septum and crimp cap, and seal. Heat the sealed vial at 90° for 2 minutes, and shake for 6 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector, a 0.53-mm × 30-m fused silica column coated with a 3.0-µm film of stationary phase G43, and a fused silica precolumn. Helium is used as the carrier gas at a flow rate of about 7 mL per minute. The injection port and detector temperatures are maintained at about 170° and 250°, respectively. Condition the column with the helium flowing at 200° for 2 hours or until a stable baseline is obtained. For analysis, the column temperature is programmed according to the following steps. It is maintained at 35° for 3 minutes, then increased to 90° at a rate of 20° per minute, then increased further to 200° at a rate of 40° per minute, and then maintained for 2 minutes. Chromatograph the headspace of the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.5 for methanol, 0.6 for ethanol, and 1.0 for *n*-propyl alcohol; the resolution, *R,* between the methanol peak and the ethanol peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 5%.

Procedure—Use a heated gas tight syringe to make injections of the headspace into the chromatograph. Separately inject equal volumes (about 1 mL) of the headspace of the *Standard solution,* the *Blank,* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the percentage of methanol and ethanol in the Ofloxacin taken by the formula:

$$(2/W)(R_U - R_B)/(R_S - R_B),$$

in which W is the weight, in mg, of Ofloxacin taken to prepare the *Test solution;* and R_U, R_B, and R_S are the peak response ratios of the relevant alcohol peak to the internal standard peak obtained from the *Test solution,* the *Blank,* and the *Standard solution,* respectively: not more than 0.005% of methanol and not more than 0.05% of ethanol are found.

Assay—Transfer about 100 mg of Ofloxacin, accurately weighed, to a 400-mL beaker, add 275 mL of acetic anhydride, and stir to dissolve. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass-silver chloride electrode system (see *Titrimetry* ⟨541⟩). Use the first of the two inflection points. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.138 mg of $C_{18}H_{20}FN_3O_4$.

Ofloxacin Ophthalmic Solution

» Ofloxacin Ophthalmic Solution is a sterile aqueous solution of Ofloxacin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ofloxacin ($C_{18}H_{20}FN_3O_4$).

Packaging and storage—Preserve in tight containers at controlled room temperature.

USP Reference standards ⟨11⟩—*USP Ofloxacin RS.*

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution—Dilute a portion of Ophthalmic Solution with a mixture of chloroform and methanol (1 : 1) to obtain a solution having a concentration of about 0.3 mg of ofloxacin per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Ofloxacin RS in a mixture of chloroform and methanol (1 : 1) to obtain a solution having a concentration of 3.0 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 5 mL of water, dilute with a mixture of chloroform and methanol (1 : 1) to volume, and mix.

Application volume: 2 µL.

Developing solvent system: a mixture of chloroform, methanol, and a solution (1 in 30) of ammonium hydroxide (150 : 75 : 15). Saturate a paper-lined chromatographic chamber with this mixture.

B: The retention time of the ofloxacin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 6.0 and 6.8.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of sodium dodecyl sulfate (0.24% aqueous solution), acetonitrile, and glacial acetic acid (580 : 400 : 20). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

0.05 N Hydrochloric acid—Add 4.0 mL of hydrochloric acid to 500 mL of water, dilute with water to 1000 mL, and mix.

Resolution solution—Prepare a solution of about 0.1 mg of USP Ofloxacin RS and about 2.4 mg of propylparaben in each mL of acetonitrile.

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Ofloxacin RS in *0.05 N Hydrochloric acid* to obtain a solution having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 3 mg of ofloxacin, to a 100-mL volumetric flask, dilute with *0.05 N Hydrochloric acid* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 294-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at a constant temperature of about 35°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between propylparaben and ofloxacin is not less than 2. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of ofloxacin ($C_{18}H_{20}FN_3O_4$) in each mL of the Ophthalmic Solution taken by the formula:

$$50(C/V)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Ofloxacin in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken to prepare the *Assay preparation;* and r_U and r_S are the ofloxacin peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Oxymetazoline Hydrochloride Ophthalmic Solution

» Oxymetazoline Hydrochloride Ophthalmic Solution is a sterile, buffered solution of Oxymetazoline Hydrochloride in water adjusted to a suitable tonicity. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{24}N_2O \cdot HCl$. It contains a suitable preservative.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Oxymetazoline Hydrochloride RS.*

Identification—A volume of Ophthalmic Solution, equivalent to about 2.5 mg of oxymetazoline hydrochloride, responds to the *Identification* test under *Oxymetazoline Hydrochloride Nasal Solution.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.8 and 6.8.

Assay—

Mobile phase—Prepare as directed in the *Assay* under *Oxymetazoline Hydrochloride.*

Standard preparation—Prepare a solution of USP Oxymetazoline Hydrochloride RS in *Mobile phase*, having a known concentration approximately equal to the labeled concentration of the Ophthalmic Solution.

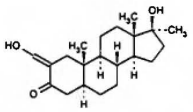
Assay preparation—Use Ophthalmic Solution.

Chromatographic system and *Procedure*—Proceed as directed in the *Assay* under *Oxymetazoline Hydrochloride*, except to calculate the quantity, in mg, of $C_{16}H_{24}N_2O \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

$$C(r_U / r_S),$$

in which the terms are as defined therein.

Oxymetholone



$C_{21}H_{32}O_3$ 332.48

Androstan-3-one, 17-hydroxy-2-(hydroxymethylene)-17-methyl-, (5α,17β)-.

17β-Hydroxy-2-(hydroxymethylene)-17-methyl-5α-androstan-3-one [*434-07-1*].

» Oxymetholone contains not less than 97.0 percent and not more than 103.0 percent of $C_{21}H_{32}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP Oxymetholone RS.*

Completeness of solution—Dissolve 100 mg in 5 mL of dioxane: the solution is clear and free from undissolved solid.

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: 0.01 N methanolic sodium hydroxide.

Melting range ⟨741⟩: between 172° and 180°.

Specific rotation ⟨781S⟩: between +34° and +38°.

Test solution: 20 mg per mL, in dioxane.

Loss on drying ⟨731⟩—Dry it in vacuum over phosphorus pentoxide for 4 hours: it loses not more than 1.0% of its weight.

Organic volatile impurities, *Method V* ⟨467⟩: meets the requirements.

Solvent—Use dimethyl sulfoxide.

Assay—

Standard preparation—Prepare as directed under *Single-steroid Assay* ⟨511⟩, using USP Oxymetholone RS.

Assay preparation—Weigh accurately about 20 mg of Oxymetholone, previously dried, dissolve in a sufficient quantity of a mixture of equal volumes of alcohol and chloroform to make 10.0 mL, and mix.

Procedure—Proceed as directed for *Procedure* under *Single-steroid Assay* ⟨511⟩, using a solvent system consisting of a mixture of benzene and alcohol (98 : 2), through the fourth sentence of the second paragraph under *Procedure.* Then centrifuge the tubes for 5 minutes, and determine the absorbances of the supernatants in 1-cm cells at the wavelength of maximum absorbance at about 315 nm, with a suitable spectrophotometer, against the blank. [NOTE—Use 0.01 *N* alcoholic sodium hydroxide, rather than alcohol, to elute the silica gel bands.] Calculate the quantity, in mg, of $C_{21}H_{32}O_3$ in the portion of Oxymetholone taken by the formula:

$$10C(A_U / A_S),$$

in which C is the concentration, in mg per mL, of USP Oxymetholone RS in the *Standard preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Oxymetholone Tablets

» Oxymetholone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{21}H_{32}O_3$.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP Oxymetholone RS.*

Identification—Mix an amount of powdered Tablets, equivalent to about 50 mg of oxymetholone, with 15 mL of solvent hexane, and stir occasionally for 15 minutes. Centrifuge the mixture, and decant and discard the solvent hexane. Extract the residue with two 10-mL portions of solvent hexane, centrifuging and decanting as before, and discard the solvent hexane. Add 25 mL of chloroform to the residue, mix by shaking for 1 to 2 minutes, and filter. Evaporate the filtrate to about 3 mL, add a few mL of solvent hexane to induce crystallization, and evaporate to dryness: the IR absorption spectrum of a potassium bromide dispersion prepared from the oxymetholone so obtained, and previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Oxymetholone RS, crystallized from the same solvent mixture.

Dissolution ⟨711⟩—

Medium: 0.05 M pH 8.5 alkaline borate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{21}H_{32}O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 313 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium* if necessary, in comparison with a Standard solution having a known concentration of USP Oxymetholone RS in the same medium. [NOTE—An amount of acetonitrile not to exceed 5% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Dissolution Medium.*]

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{32}O_3$ is dissolved in 45 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of about 75 mL of methanol. Heat the methanol to boiling, and allow to remain at a temperature just below the boiling point for 15 minutes with

Standard preparation—Dissolve about 50 mg of USP Phenylephrine Hydrochloride RS, accurately weighed, in 10 mL of water, dilute with *Dilution solvent* to 25.0 mL, and mix. Further dilute 5.0 mL of the resulting solution with *Dilution solvent* to 25.0 mL, and mix to obtain a solution having a known concentration of about 0.4 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of phenylephrine hydrochloride, to a 25-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the responses for the major peaks: the resolution, *R*, between epinephrine and phenylephrine is not less than 1.0. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(25C/V)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Nasal Jelly

» Phenylephrine Hydrochloride Nasal Jelly contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Phenylephrine Hydrochloride RS.*

Identification—Dissolve a suitable quantity in water to obtain a solution having a concentration of about 60 µg per mL, and centrifuge, if necessary: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Phenylephrine Hydrochloride RS, concomitantly measured.

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase—Prepare a mixture of methanol and water (1 : 1) containing 1.1 g of sodium 1-octanesulfonate per liter, adjust with phosphoric acid to a pH of 3.0, filter, and degas. Make adjustments to the methanol and water ratio, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Dilution solvent—Prepare a mixture of methanol and water (1 : 1), and adjust with phosphoric acid to a pH of 3.0.

Standard preparation—Dissolve an accurately weighed quantity of USP Phenylephrine Hydrochloride RS in *Dilution solvent* to obtain a Stock standard solution having a known concentration of about 2 mg per mL. Dilute an accurately measured volume of this solution with *Dilution solvent* to obtain the *Standard preparation* having a known concentration of about 0.1 mg per mL.

Assay preparation—Transfer an accurately weighed amount of Nasal Jelly, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Resolution solution—Transfer 5.0 mL of Stock standard solution to a 100-mL volumetric flask, add 10 mg of USP Epinephrine Bitartrate RS, dilute with *Dilution solvent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution:* the resolution, *R*, is not less than 1.5, and the tailing factor for the phenylephrine peak is not more than 2.0. Chromatograph replicate injections of the *Standard preparation:* the relative standard deviation is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in the portion of Nasal Jelly taken by the formula:

$$100C(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Nasal Solution

» Phenylephrine Hydrochloride Nasal Solution contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Phenylephrine Hydrochloride RS.*

Identification—It responds to the *Identification* test under *Phenylephrine Hydrochloride Injection.*

Assay—

Mobile phase, Dilution solvent, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Phenylephrine Hydrochloride Nasal Jelly.*

Assay preparation—Transfer an accurately measured volume of Nasal Solution, equivalent to about 10 mg of phenylephrine hydrochloride, to a 100-mL volumetric flask. Dilute with *Dilution solvent* to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}NO_2 \cdot HCl$ in each mL of the Nasal Solution taken by the formula:

$$100(C/V)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*, V is the volume, in mL, of Nasal Solution taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Phenylephrine Hydrochloride Ophthalmic Solution

» Phenylephrine Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Phenylephrine Hydrochloride. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_9H_{13}NO_2 \cdot HCl$. It may contain a suitable antimicrobial agent and buffer and may contain suitable antioxidants.

Packaging and storage—Preserve in tight, light-resistant containers of not more than 15-mL size.

USP Reference standards ⟨11⟩—*USP Phenylephrine Hydrochloride RS.*

Organic volatile impurities, *Method IV* ⟨467⟩: meets the requirements.

Phenylpropanolamine Bitartrate

$C_9H_{13}NO \cdot C_4H_6O_6$ 301.30
(*R**,*S**)-(±)-α-(1-Aminoethyl)benzenemethanol bitartrate [*67244-90-0*].

» Phenylpropanolamine Bitartrate contains not less than 98.0 percent and not more than 101.0 percent of $C_9H_{13}NO \cdot C_4H_6O_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Cathinone Hydrochloride RS. USP Dextroamphetamine Sulfate RS. USP Phenylpropanediol RS. USP Phenylpropanolamine Bitartrate RS. USP Phenylpropanolamine Hydrochloride RS.*

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: It responds to the test for *Tartrate* ⟨191⟩.

Melting range, *Class I* ⟨741⟩: between 150° and 164°.

pH ⟨791⟩: between 3.1 and 3.7, in a solution (3 in 100).

Loss on drying ⟨731⟩—Dry it at 65° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method I* ⟨231⟩—Dissolve 1 g in 5 mL of water, add 1 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is 0.002%.

Limit of cathinone hydrochloride—Proceed as directed for *Limit of cathinone hydrochloride* under *Phenylpropanolamine Hydrochloride.*

Limit of amphetamine hydrochloride and phenylpropanediol—

Mobile phase—Prepare a mixture of 20 mL of 10% tetramethylammonium hydroxide and 5 mL of phosphoric acid, dilute with water to a volume of 1000 mL, and mix. To 896 mL of the resulting solution add 100 mL of methanol, 4 mL of tetrahydrofuran, and mix. Filter and degas the mixture. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution A—Dissolve accurately weighed quantities of USP Phenylpropanolamine Hydrochloride RS and USP Dextroamphetamine Sulfate RS in water to obtain a solution having known concentrations of about 100 mg of USP Phenylpropanolamine Hydrochloride RS per mL and 1 µg of USP Dextroamphetamine Sulfate RS per mL.

Standard solution B—Dissolve an accurately weighed quantity of USP Phenylpropanediol RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per mL.

Resolution solution—Dissolve accurately weighed quantities of USP Phenylpropanolamine Hydrochloride RS and USP Dextroamphetamine Sulfate RS in water to obtain a solution containing about 5 µg of each per mL.

Test solution—Transfer about 1000 mg of Phenylpropanolamine Bitartrate, accurately weighed, to a 10-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains spherical 5-µm packing L1. The flow rate is about 2.0 mL per minute. Separately chromatograph the *Resolution solution* and each *Standard solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for phenylpropanolamine, between 1.9 and 2.1 for dextroamphetamine, and between 2.3 and 2.7 for phenylpropanediol; the resolution, *R*, between phenylpropanolamine and dextroamphetamine in the chromatogram of the *Resolution solution* is not less than 5.0; and the relative standard deviation for replicate injections of the *Standard solutions* is not more than 3.0%.

Procedure—Separately inject equal volumes (about 20 µL) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the

USP Reference standards ⟨11⟩—*USP Physostigmine Salicylate RS. USP Endotoxin RS.*

Identification—

A: It responds to the *Identification* test under *Physostigmine.*

B: It responds to the tests for *Salicylate* ⟨191⟩.

Bacterial endotoxins ⟨85⟩—It contains not more than 83.4 USP Endotoxin Units per mg of physostigmine salicylate.

pH ⟨791⟩: between 3.5 and 5.0.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

0.05 M Ammonium acetate—Dissolve 3.85 g of ammonium acetate in 1 liter of water, and adjust, if necessary, with glacial acetic acid or ammonium hydroxide to a pH of 6 ± 0.1.

Mobile phase—Prepare a filtered and degassed mixture of equal volumes of acetonitrile and *0.05 M Ammonium acetate.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Benzyl alcohol-benzaldehyde solution—Prepare a mixture of 100 µL of benzyl alcohol and 1 µL of benzaldehyde in each 400 mL of acetonitrile.

Standard preparation—Dissolve an accurately weighed quantity of USP Physostigmine Salicylate RS in *Benzyl alcohol-benzaldehyde solution*, and dilute quantitatively, and stepwise if necessary, with *Benzyl alcohol-benzaldehyde solution*, to obtain a solution having a known concentration of about 30 µg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 3 mg of physostigmine salicylate, to a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Separately chromatograph 10-µL portions of the *Benzyl alcohol-benzaldehyde solution* and the *Standard preparation*, and record the peak responses as directed under *Procedure* [NOTE—If the components of the *Benzyl alcohol-benzaldehyde solution* co-elute, the *Standard preparation* will exhibit only 2 peaks instead of 3.]: in a suitable system, benzyl alcohol and benzaldehyde elute before physostigmine, the column efficiency determined from the analyte peak is not less than 1200 theoretical plates, the resolution, *R*, between physostigmine and the adjacent peak (benzyl alcohol or benzaldehyde or the combination of these) is not less than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$ in each mL of the Injection taken by the formula:

$$0.1(C/V)(r_U/r_S),$$

in which C is the concentration, in µg per mL, of USP Physostigmine Salicylate RS in the *Standard preparation*, V is the volume, in mL, of Injection taken, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Physostigmine Salicylate Ophthalmic Solution

» Physostigmine Salicylate Ophthalmic Solution is a sterile, aqueous solution of Physostigmine Salicylate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$. It may contain suitable antimicrobial agents, buffers, and stabilizers, and suitable additives to increase its viscosity.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Physostigmine Salicylate RS.*

Identification—It responds to the *Identification* tests under *Physostigmine Salicylate.*

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 2.0 and 4.0.

Assay—

0.05 M Ammonium acetate and *Mobile phase*—Prepare as directed in the *Assay* under *Physostigmine Salicylate Injection.*

Standard preparation—Dissolve an accurately weighed quantity of USP Physostigmine Salicylate RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile, to obtain a solution having a known concentration of about 30 µg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 3 mg of physostigmine salicylate, to a 100-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1200 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Physostigmine Salicylate Injection.* Calculate the quantity, in mg, of $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.1(C/V)(r_U/r_S),$$

in which the terms are as defined therein.

Physostigmine Sulfate

$(C_{15}H_{21}N_3O_2)_2 \cdot H_2SO_4$ 648.77

Pyrrolo[2,3-*b*]indol-5-ol, 1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethyl-, methylcarbamate (ester), (3a*S-cis*)-, sulfate (2 : 1).

Physostigmine sulfate (2 : 1) [*64-47-1*].

» Physostigmine Sulfate contains not less than 97.0 percent and not more than 102.0 percent of $(C_{15}H_{21}N_3O_2)_2 \cdot H_2SO_4$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩—*USP Physostigmine Salicylate RS.*

Identification—

A: It responds to the *Identification* test under *Physostigmine.*

B: A solution (1 in 100) responds to the tests for *Sulfate* ⟨193⟩.

Specific rotation ⟨781S⟩: between −116° and −120°.

Test solution: 10 mg per mL, in water.

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: negligible, from 100 mg.

Readily carbonizable substances—It meets the requirements of the test for *Readily carbonizable substances* under *Physostigmine.*

Assay—Dissolve about 200 mg of Physostigmine Sulfate, accurately weighed, in 25 mL of water. Render the solution alkaline by the addition of about 1 g of sodium bicarbonate, and extract with one 25-mL and five 10-mL portions of chloroform, each time shaking vigorously for 1 minute. Filter each extract through glass wool, add 15 mL of glacial acetic acid and 10 mL of acetic acid anhydride to the combined chloroform extracts, and titrate with 0.02 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.02 N perchloric acid is equivalent to 6.488 mg of $(C_{15}H_{21}N_3O_2)_2 \cdot H_2SO_4$.