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USP 28

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NF 23

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apraclonidine peak, in the specimen of Apraclonidine Hydrochloride taken by the same formula:

$$100r_i/r_t$$

in which r_i is the response of each peak other than the principal peak, and r_t is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% for any individual impurity and not more than 2.0% total impurities are found.

Assay—Dissolve about 125 mg of Apraclonidine Hydrochloride, accurately weighed, in 40 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically from the second inflection point, using a calomel-glass electrode system (see *Titrimetry* ⟨541⟩). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.08 mg of $C_9H_{10}Cl_2N_4 \cdot HCl$.

Apraclonidine Ophthalmic Solution

» Apraclonidine Ophthalmic Solution is a sterile, aqueous solution of Apraclonidine Hydrochloride. It contains an amount of apraclonidine hydrochloride ($C_9H_{10}Cl_2N_4 \cdot HCl$) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of apraclonidine ($C_9H_{10}Cl_2N_4$).

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

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

Identification—

A: 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.*

B: Apply 2 µL of Apraclonidine Ophthalmic Solution and 2 µL of a Standard solution of USP Apraclonidine Hydrochloride RS in methanol containing about 11.5 mg per mL to a suitable high performance thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.2-mm layer of chromatographic silica gel mixture, or equivalent. Allow the applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (74 : 22 : 4) 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 viewing under short-wavelength UV light. [NOTE—The apraclonidine spot should appear as a blue spot.] Spray the plate with fluorescamine solution, prepared by dissolving about 25 mg of fluorescamine in 25 mL of acetone. [NOTE—Avoid prolonged or repeated breathing of the aerosol from the fluorescamine spray. Also avoid prolonged or repeated contact with skin. Fluorescamine solution should be sprayed only in a hood.] Examine the plate under normal light and long-wavelength UV light. [NOTE—The apraclonidine spot should appear as a yellow spot under normal light and as a white spot under long-wavelength UV light.] The R_F value and appearance 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 4.4 and 7.8.

Assay—

Phosphate buffer—Prepare as directed in the test for *Chromatographic purity* under *Apraclonidine Hydrochloride.*

Mobile phase—Prepare a filtered and degassed mixture of *Phosphate buffer*, acetonitrile, and methanol (68 : 30 : 2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Apraclonidine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a *Stock standard solution* having a known concentration of about 0.23 mg per mL. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 11.5 µg of USP Apraclonidine Hydrochloride RS per mL (equivalent to about 10 µg of apraclonidine per mL).

Resolution solution—Transfer about 1 mL of propiophenone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Stock standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of apraclonidine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

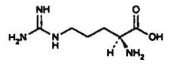
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.6 for apraclonidine and 1.0 for propiophenone; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; the resolution, R, between the analyte and propiophenone peaks is not less than 3.0; 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 apraclonidine ($C_9H_{10}Cl_2N_4$) in each mL of the Ophthalmic Solution taken by the formula:

$$(245.11/281.57)(2C/V)(r_U/r_S),$$

in which 245.11 and 281.57 are the molecular weights of apraclonidine and apraclonidine hydrochloride, respectively; C is the concentration, in µg per mL, of USP Apraclonidine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the apraclonidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Arginine



$C_6H_{14}N_4O_2$ 174.20

L-Arginine.

L-Arginine [*74-79-3*].

» Arginine contains not less than 98.5 percent and not more than 101.5 percent of $C_6H_{14}N_4O_2$, as L-arginine, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP L-Arginine RS. USP L-Lysine Hydrochloride RS.*

Identification, *Infrared Absorption* ⟨197K⟩.

Specific rotation ⟨781S⟩: between +26.3° and +27.7°.

Test solution: 80 mg per mL, in 6 N hydrochloric acid.

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

Residue on ignition ⟨281⟩: not more than 0.3%.

Chloride ⟨221⟩—A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).

Other alkaloids—Dissolve 150 mg in 10 mL of water. To 5 mL of the solution add a few drops of platinic chloride TS: no precipitate is formed. To the remaining 5 mL of the solution add 2 mL of 6 N ammonium hydroxide, and shake vigorously: a slight opalescence may develop but no turbidity is produced.

Assay—Dissolve about 1 g of Atropine Sulfate, accurately weighed, in 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 67.68 mg of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$.

Atropine Sulfate Injection

» Atropine Sulfate Injection is a sterile solution of Atropine Sulfate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$.

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

USP Reference standards ⟨11⟩—*USP Atropine Sulfate RS. USP Endotoxin RS.*

Identification (see *Thin-Layer Chromatographic Identification Test* ⟨201⟩)—

Adsorbent: chromatographic silica gel.

Developing solvent: mixture of chloroform and diethylamine (9 : 1).

Test preparation—Use undiluted. Apply 15 µL.

Detection reagent: potassium iodoplatinate TS.

Procedure—Proceed as directed for *Procedure* under *Thin-Layer Chromatographic Identification Test* ⟨201⟩, the spots on the plate located by spraying with *Detection reagent.*

Bacterial endotoxins ⟨85⟩—It contains not more than 55.6 USP Endotoxin Units per mg of atropine sulfate.

pH ⟨791⟩: between 3.0 and 6.5.

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

Assay—

Acetate buffer—Prepare a solution in water containing in each L 0.05 mole of sodium acetate and 2.9 mL of glacial acetic acid.

Mobile phase—Transfer 5.1 g of tetrabutylammonium hydrogen sulfate to a 1-L volumetric flask, add 50 mL of acetonitrile, and dilute with *Acetate buffer* to volume. Adjust with 5 N sodium hydroxide to a pH of 5.5 ± 0.1.

Standard preparation—Dissolve an accurately weighed quantity of USP Atropine Sulfate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 80 µg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 2 mg of atropine sulfate, to a 25-mL volumetric flask, dilute with water to volume, and mix.

Resolution solution—Prepare a solution in water containing about 2.5 µg of *p*-hydroxybenzoic acid per mL. Dilute one volume of this solution with four volumes of the *Standard preparation.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and 30-cm × 3.9-mm 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 relative standard deviation for replicate injections is not more than 1.5%. In a similar manner, chromatograph the *Resolution solution:* the retention time of *p*-hydroxybenzoic acid is about 1.6 relative to that of atropine, and the resolution, *R,* between the *p*-hydroxybenzoic acid and atropine peaks is not less than 2.2.

Procedure—Separately inject equal volumes (about 100 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 $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ in each mL of the Injection taken by the formula:

$$(694.85/676.83)(25C/V)(r_U/r_S),$$

in which 694.85 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; C is the concentration, in mg per mL, of USP Atropine Sulfate 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.

Atropine Sulfate Ophthalmic Ointment

» Atropine Sulfate Ophthalmic Ointment is Atropine Sulfate in a suitable ophthalmic ointment base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$. It is sterile.

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes.

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

Identification—

A: Transfer a portion of Ophthalmic Ointment, equivalent to about 50 mg of atropine sulfate, to a suitable separator, and dissolve in 25 mL of ether. Add 25 mL of 0.01 N hydrochloric acid, shake vigorously, allow the layers to separate, and discard the organic phase. Heat the aqueous phase gently on a steam bath while passing nitrogen through the solution, to expel any residual ether. Proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "In a second separator dissolve 50 mg."

B: Transfer about 5 g of Ophthalmic Ointment to a separator, dissolve in 50 mL of ether, and extract with 20 mL of water: the extracted solution so obtained responds to the tests for *Sulfate* ⟨191⟩.

Sterility ⟨71⟩: It meets the requirements.

Metal particles ⟨751⟩: It meets the requirements.

Assay—Proceed with Ophthalmic Ointment as directed in the *Assay* under *Atropine Sulfate Ophthalmic Solution,* but in preparing the *Assay preparation,* weigh accurately a portion of Ophthalmic Ointment, equivalent to about 10 mg of atropine sulfate, transfer to a separator containing 50 mL of ether, shake to dissolve, extract with three 25-mL portions of 0.1 M sulfuric acid, collect the acid extracts in a 100-mL volumetric flask, dilute with 0.1 M sulfuric acid to volume, and mix. Proceed as directed for the *Assay preparation* in the *Assay* under *Atropine Sulfate Ophthalmic Solution,* beginning with "Pipet 10 mL of this solution into a separator." Calculate the quantity, in mg, of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of Ophthalmic Ointment taken by the formula given therein.

Atropine Sulfate Ophthalmic Solution

» Atropine Sulfate Ophthalmic Solution is a sterile, aqueous solution of Atropine Sulfate. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$. It may contain suitable stabilizers and antimicrobial agents.

Packaging and storage—Preserve in tight containers.

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

Identification—After evaporation to dryness, it meets the requirements for *Identification* test *A* under *Atropine* and for *Identification* test *B* under *Atropine Sulfate.*

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Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 3.5 and 6.0.

Assay—

pH 9.0 Buffer—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.0, determined electrometrically, by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide, as necessary, with mixing.

Internal standard solution—[NOTE—Prepare fresh daily.] Transfer about 25 mg of homatropine hydrobromide to a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Standard preparation—[NOTE—Prepare fresh daily.] Dissolve an accurately weighed quantity of USP Atropine Sulfate 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. Pipet 10 mL of this solution into a separator, and proceed as directed for the *Assay preparation*, beginning with "Add 2.0 mL of *Internal standard solution*."

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of Atropine Sulfate, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 10 mL of this solution and treat as follows. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *pH 9.0 Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 1.8-m glass column packed with a 3% phase G3 on support S1AB. The carrier gas is nitrogen, flowing at a rate of 25 mL per minute. The column temperature is maintained at 225°. The injection port and detector temperatures are maintained at 250°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the resolution, R, is not less than 4.0; 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 1 µL) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in each mL of Ophthalmic Solution taken by the formula:

$$(694.85/676.83)(W/V)(R_U/R_S),$$

in which 694.85 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; W is the weight, in mg, of USP Atropine Sulfate RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken; and R_U and R_S are the peak area ratios of atropine sulfate to homatropine hydrobromide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Atropine Sulfate Tablets

» Atropine Sulfate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$.

Packaging and storage—Preserve in well-closed containers.

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

Identification—

A: Triturate a quantity of Tablets, equivalent to about 5 mg of atropine sulfate, with 10 mL of water for a few minutes, and filter into a small separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract with 50 mL of chloroform. Filter the chloroform layer, and evaporate to dryness. The residue so obtained meets the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

B: A filtered solution of Tablets responds to the tests for *Sulfate* ⟨191⟩.

Disintegration ⟨701⟩: 15 minutes.

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

Assay—

pH 9.0 Buffer, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Atropine Sulfate Ophthalmic Solution.*

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of atropine sulfate, to a separator, and proceed as directed for the *Assay preparation* in the *Assay* under *Atropine Sulfate Ophthalmic Solution*, beginning with "Add 2.0 mL of *Internal standard solution.*"

Procedure—Proceed as directed in the *Assay* under *Atropine Sulfate Ophthalmic Solution.* Calculate the quantity, in mg, of atropine sulfate $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ in the portion of Tablets taken by the formula:

$$(694.85/676.83)(W/10)(R_U/R_S),$$

in which 694.85 and 676.83 are the molecular weights of atropine sulfate monohydrate and anhydrous atropine sulfate, respectively; W is the weight, in mg, of USP Atropine Sulfate RS in the *Standard preparation;* and R_U and R_S are as defined therein.

Activated Attapulgite

» Activated Attapulgite is a highly heat-treated, processed, native magnesium aluminum silicate.

Packaging and storage—Preserve in well-closed containers.

Identification—Activated Attapulgite responds to the *Identification* test for *Colloidal Activated Attapulgite*, the characteristic peak, however, being much less intense.

Loss on drying ⟨731⟩—Dry it at 105° to constant weight: it loses not more than 4.0% of its weight.

Loss on ignition ⟨733⟩—When ignited at 1000° for 1 hour, it loses between 4.0% and 12.0% of its weight.

Volatile matter—When ignited at 600° for 1 hour, it loses between 3.0% and 7.5% of its weight on the dried basis.

Powder fineness—Proceed as directed in the test for *Powder fineness* under *Colloidal Activated Attapulgite.* The dry weight of the residue is not more than 0.10% of the weight of the specimen taken.

Acid-soluble matter—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.25 g is found (25%).

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

Other requirements—It meets the requirements of the tests for *Microbial limits, pH, Carbonate, Arsenic and Lead,* and *Adsorptive capacity* under *Colloidal Activated Attapulgite.*

Colloidal Activated Attapulgite

» Colloidal Activated Attapulgite is a purified native magnesium aluminum silicate.

Packaging and storage—Preserve in well-closed containers.

Identification—Add 2 g in small portions to 100 mL of water, with vigorous agitation. Allow to stand for at least 12 hours to ensure complete hydration. Place 2 mL of the resulting mixture on a suitable glass slide, and allow to air-dry at room temperature to produce a uniform film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that the ethylene glycol saturates the desiccator chamber. Allow to stand for 12 hours. Record the X-ray diffraction pattern (see *X-ray Diffraction* ⟨941⟩), and calculate the d values: several peaks are

observed; the cha
10.3 and 10.7 An

Microbial limits
absence of *Escher*

pH ⟨791⟩—Dispe
mix: the pH of t
9.5.

Loss on drying ⟨
between 5.0% and

Loss on ignition
between 17.0% an

Volatile matter—
7.5% and 12.5% o

Powder fineness—
sodium pyrophosp
dispersion slowly t
Distribution Estim
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weight: the dry we
0.30% of the weigh

Acid-soluble matt
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mL, and filter. Eva
and ignite the resid

Carbonate—Mix
effervescence occu

Arsenic and Lead
for 30 minutes, addi
Filter into a 100-mL
dilute the combinec

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directed by the man
the absorbance at 18
found.

Lead—Determine
spectrometry (see S
using a graphite fu
manufacturer of the
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Adsorptive capacit
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and shake. Add 10
shake. Allow to st
supernatant to a 50-
the clear supernatant
solution so obtained
1.5 µg of methylene

Organic volatile im
ments.

Aurothioglu

$C_6H_{11}AuO_5S$ 392.18
Gold, (1-thio-D-gluco
(1-Thio-D-glucopyran

Bendroflumethiazide Tablets

» Bendroflumethiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{15}H_{14}F_3N_3O_4S_2$.

Packaging and storage—Preserve in tight containers.

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

Identification—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⟩—[NOTE—Protect solutions from light throughout this test.]

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

Procedure—Determine the amount of $C_{15}H_{14}F_3N_3O_4S_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 271 nm on filtered portions of the solution under test, suitably diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Bendroflumethiazide RS in the same *Medium.*

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{15}H_{14}F_3N_3O_4S_2$ is dissolved in 45 minutes.

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

Assay—[NOTE—Use low-actinic glassware for the *Assay preparation* and the *Standard preparation.*]

Mobile phase—Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium acetate in 1000 mL of water in a 2-liter volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, dilute with water to volume, mix, filter, and degas.

Standard preparation—Dissolve an accurately weighed quantity of USP Bendroflumethiazide RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 50 µg per mL.

Assay preparation—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 5 mg of bendroflumethiazide, to a 100-mL volumetric flask, add about 70 mL of methanol, and sonicate for 15 minutes, with occasional shaking. Dilute with methanol to volume, mix, and centrifuge a portion of the solution for 15 minutes.

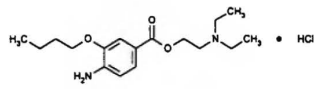
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 30-cm column that contains packing L11 maintained at a temperature of 35 ± 5°. The flow rate is about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%, and the tailing factor 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 response of the major peak. Calculate the quantity, in mg, of $C_{15}H_{14}F_3N_3O_4S_2$ in the portion of Tablets taken by the formula:

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

in which C is the concentration, in µg per mL, of USP Bendroflumethiazide 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.

Benoxinate Hydrochloride



$C_{17}H_{28}N_2O_3 \cdot HCl$ 344.88

Benzoic acid, 4-amino-3-butoxy-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate monohydrochloride [*5987-82-6*].

» Benoxinate Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of $C_{17}H_{28}N_2O_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

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

Identification—

A: *Infrared Absorption* ⟨197K⟩: previously dried.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 15 µg per mL.

Medium: water.

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

pH ⟨791⟩: between 5.0 and 6.0, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Ordinary impurities ⟨466⟩—

Test solution: methanol.

Standard solution: methanol.

Eluant: a mixture of chloroform, cyclohexane, and diethylamine (5 : 4 : 1).

Visualization: 12.

Assay—Dissolve about 250 mg of Benoxinate Hydrochloride, accurately weighed, in a mixture of 20 mL of glacial acetic acid and 20 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.49 mg of $C_{17}H_{28}N_2O_3 \cdot HCl$.

Benoxinate Hydrochloride Ophthalmic Solution

» Benoxinate Hydrochloride Ophthalmic Solution is a sterile solution of Benoxinate Hydrochloride in water. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{17}H_{28}N_2O_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

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

Identification—Dilute a volume of Solution, equivalent to about 50 mg of benoxinate hydrochloride, with 0.01 N hydrochloric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator": the solution meets the requirements of the test.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 3.0 and 6.0.

Assay—

Standard prepar
USP Benoxinate H
obtain a solution h
mL.

Assay preparati
equivalent to about
containing 15 mL
extract with five 2
extracts with 10 m
of ether, and add
ether solution with
collect the acid extr
hydrochloric acid t

Procedure—Trar
Assay preparation,
separate 200-mL v
with water to vo
absorbances of th
maximum absorba
tometer, using the
in mg, of $C_{17}H_{28}N$
taken by the formu

in which *C* is the
Hydrochloride RS
mL, of Ophthalmic
of the solutions
preparation, respe

Benzethoni

$C_{27}H_{42}ClNO_2$ 44
Benzenemethanan
methylbutyl)p
Benzyldimethyl[2
oxy]ethyl]am

» Benzethoniu
percent and
$C_{27}H_{42}ClNO_2$,

Packaging and st
Identification—
A: To 1 mL of
of 2 N nitric acid,
which is insoluble
hydroxide, is form
B: A solution
and with mercuric
addition of alcoho
C: Dissolve
potassium nitrate,
dilute the solution
and warm the mix
nitrite to 1 mL of
naphthol dipotassiu
1 mL of ammoniu
brown precipitate
Melting range ⟨74
been dried previou
Loss on drying ⟨7
than 5.0% of its w

Assay—

Standard preparation—Dissolve an accurately weighed quantity of USP Benoxinate Hydrochloride RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 400 µg per mL.

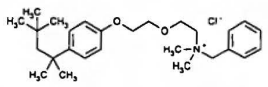
Assay preparation—Transfer a volume of Ophthalmic Solution, equivalent to about 20 mg of benoxinate hydrochloride, to a separator containing 15 mL of water, add 1 mL of ammonium hydroxide, and extract with five 20-mL portions of ether. Wash the combined ether extracts with 10 mL of water, extract the water washing with 10 mL of ether, and add this ether extract to the main extract. Extract the ether solution with three 5-mL portions of 0.1 N hydrochloric acid, collect the acid extracts in a 50-mL volumetric flask, dilute with 0.1 N hydrochloric acid to volume, and mix.

Procedure—Transfer 5.0 mL each of the *Standard preparation*, the *Assay preparation*, and 0.1 N hydrochloric acid to provide a blank, to separate 200-mL volumetric flasks. Dilute the contents of each flask with water to volume, and mix. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 308 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of $C_{17}H_{28}N_2O_3 \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

$$(0.05C/V)(A_U/A_S),$$

in which C is the concentration, in µg per mL, of USP Benoxinate Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Benzethonium Chloride



$C_{27}H_{42}ClNO_2$ 448.08

Benzenemethanaminium, *N,N*-dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride.

Benzyldimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride [*121-54-0*].

» Benzethonium Chloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{27}H_{42}ClNO_2$, calculated on the dried basis.

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

Identification—

A: To 1 mL of a solution (1 in 100) add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS: a white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

B: A solution (1 in 100) forms precipitates with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

C: Dissolve 0.1 g in 1 mL of sulfuric acid, add 0.1 g of potassium nitrate, and heat on a steam bath for 3 minutes. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 minutes. Cool, add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide: the solution turns orange-red, and a brown precipitate may be formed.

Melting range ⟨741⟩: between 158° and 163°, the specimen having been dried previously.

Loss on drying ⟨731⟩—Dry it at 105° for 4 hours: it loses not more than 5.0% of its weight.

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

Limit of ammonium compounds—To 5 mL of a solution (1 in 50) add 3 mL of 1 N sodium hydroxide, and heat to boiling: the odor of ammonia is not perceptible.

Assay—Dissolve about 0.3 g of Benzethonium Chloride, accurately weighed, in 75 mL of water contained in a glass-stoppered, 250-mL flask. Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of $C_{27}H_{42}ClNO_2$.

Benzethonium Chloride Concentrate

» Benzethonium Chloride Concentrate contains not less than 94.0 percent and not more than 106.0 percent of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

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

Labeling—The label states that this article is not intended for direct administration to humans or animals.

Identification—Evaporate a volume of Concentrate, equivalent to about 200 mg of benzethonium chloride, on a steam bath: the residue so obtained meets the requirements of the tests for *Identification* under *Benzethonium Chloride*.

Oxidizing substances—To 5 mL of Concentrate add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid: the solution does not acquire a yellow color.

Limit of nitrites—To 1 drop of Concentrate on a spot plate add 1 drop each of glacial acetic acid, sulfanilic acid in acetic acid solution (1 in 100), and 1-naphthylamine–acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid): no red color develops in the resulting solution within 10 minutes.

Assay—Transfer a volume of Concentrate, equivalent to about 200 mg of benzethonium chloride, to a glass-stoppered flask, and proceed as directed in the *Assay* under *Benzethonium Chloride*, beginning with "Add 0.4 mL of bromophenol blue solution (1 in 2000)."

Benzethonium Chloride Topical Solution

» Benzethonium Chloride Topical Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

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

Identification—The residue obtained by evaporating, on a steam bath, a volume of Topical Solution, equivalent to about 200 mg of benzethonium chloride, responds to the *Identification* tests under *Benzethonium Chloride*.

Oxidizing substances—To 5 mL add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid: the solution does not acquire a yellow color.

Limit of nitrites—To 1 drop of Topical Solution on a spot plate add 1 drop each of glacial acetic acid, sulfanilic acid in acetic acid (1 in 100), and 1-naphthylamine–acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid): no red color develops in the resulting solution within 10 minutes.

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Standard preparation—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a 1 in 1000 solution of glacial acetic acid in alcohol to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

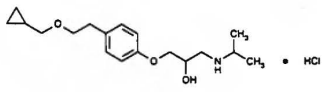
Assay preparation—Transfer an accurately weighed portion of Ointment, equivalent to about 2.5 mg of betamethasone, to a 50-mL centrifuge tube. Add 10.0 mL of the *Internal standard solution* and 5.0 mL of a 1 in 1000 solution of glacial acetic acid in alcohol. Insert the stopper into the tube, and place in a water bath held at 70° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 minutes, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Valerate*. Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in the portion of Ointment taken by the formula:

$$(392.46/476.59)(15C)(R_U/R_S),$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Betaxolol Hydrochloride



$C_{18}H_{29}NO_3 \cdot HCl$ 343.89

2-Propanol, 1-4-2-(cyclopropylmethoxy)ethylphenoxy-3-(1-methylethyl)amino-, hydrochloride, (±)-.

(±)-1-*p*-2-(Cyclopropylmethoxy)ethylphenoxy-3-(isopropylamino)-2-propanol hydrochloride [*63659-19-8*].

» Betaxolol Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of $C_{18}H_{29}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

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

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: It responds to the test for *Chloride* ⟨191⟩ when tested as directed for alkaloidal hydrochlorides.

Melting range, *Class I* ⟨741⟩: between 113° and 117°.

pH ⟨791⟩: between 4.5 and 6.5, in a solution (1 in 50).

Loss on drying ⟨731⟩—Dry it in vacuum at 65° for 2 hours: it loses not more than 1.0% of its weight.

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

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

Chromatographic purity—

Buffer—Prepare a solution of 0.025 M monobasic potassium phosphate containing 0.1% (w/v) of tetrabutylammonium bromide. Adjust with 0.025 M phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a suitable filtered and degassed mixture of *Buffer* and acetonitrile (85 : 15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Prepare a solution in *Mobile phase* containing 2.0 mg of USP Betaxolol Hydrochloride RS and 1 mg of alprenolol hydrochloride per mL.

Test preparation—Prepare a solution of Betaxolol Hydrochloride in *Mobile phase* containing 2.0 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.9 for alprenolol and 1.0 for betaxolol; the resolution, R, between the two peaks is not less than 1.0; the tailing factors for the two peaks are not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject about 20 µL of the *Test preparation* into the chromatograph, record the chromatogram, and measure the areas for the peaks. [NOTE—Allow the elution to continue for about five times the elution time of the betaxolol peak before making the next injection.] Calculate the percentage of each impurity by the same formula:

$$100(r_i/r_S),$$

in which r_i is the response of each individual peak, other than the main betaxolol peak, in the chromatogram obtained from the *Test preparation*, and r_S is the sum of the responses of all the peaks obtained in the chromatogram from the *Test preparation:* the sum of all impurities found is not more than 1.0%.

Assay—Dissolve about 300 mg of Betaxolol Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid. Add 7 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.39 mg of $C_{18}H_{29}NO_3 \cdot HCl$.

Betaxolol Ophthalmic Solution

» Betaxolol Ophthalmic Solution is a sterile, aqueous, isotonic solution of Betaxolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betaxolol ($C_{18}H_{29}NO_3$).

Packaging and storage—Preserve in tight containers.

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

Identification—Prepare a test solution by diluting a suitable volume of it with water to obtain a solution containing about 2.5 mg of betaxolol per mL. Separately apply 5 µL of the test solution and 5 µL of a Standard solution of USP Betaxolol Hydrochloride RS in water containing about 2.75 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of silica gel. Allow the spots to dry, and develop the chromatogram in a chromatographic chamber, using a solvent system consisting of a mixture of chloroform, isopropyl alcohol, and ammonium hydroxide (70 : 30 : 2) 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 plate to air-dry. Spray the plate with a 1 in 1000 solution of ninhydrin in isopropyl alcohol, and heat the plate at 105° for 10 minutes. Locate the spots on the plate: 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 4.0 and 8.0.

Assay—

pH 3.0 Buffer—Dissolve 7.1 g of anhydrous dibasic sodium phosphate in about 800 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to make 1000 mL of solution.

Mobile phase—Prepare a suitable filtered and degassed mixture of *pH 3.0 Buffer* and acetonitrile (1 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Betaxolol Hydrochloride RS in *pH 3.0 Buffer* to obtain a solution having a known concentration of about 0.11 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of betaxolol, to a 100-mL volumetric flask. Dilute with *pH 3.0 Buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1.1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the capacity factor, k', for the main betaxolol peak is between 1 and 3; the tailing factor is not less than 0.8 and not more than 2.0; the column efficiency is not less than 750 theoretical plates; 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 $C_{18}H_{29}NO_3$ in each mL of the Ophthalmic Solution taken by the formula:

$$(307.43/343.89)(100C/V)(r_U/r_S),$$

in which 307.43 and 343.89 are the molecular weights of betaxolol and betaxolol hydrochloride, respectively; C is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the *Standard preparation;* V is the volume, in mL, of Ophthalmic Solution taken; and r_U and r_S are the betaxolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Betaxolol Tablets

» Betaxolol Tablets contain an amount of Betaxolol Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$).

Packaging and storage—Preserve in tight containers.

Labeling—Label the Tablets to state both the content of the betaxolol active moiety and the content of betaxolol hydrochloride used in formulating them.

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

Identification—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: 0.01 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{18}H_{29}NO_3 \cdot HCl$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test in comparison with a Standard solution having a known concentration of USP Betaxolol Hydrochloride RS in the same *Medium.* A 5-cm pathlength cell may be used for lower dosage levels.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{18}H_{29}NO_3 \cdot HCl$ is dissolved in 30 minutes.

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

Procedure for content uniformity—Place 1 Tablet in a volumetric flask of appropriate size to obtain a concentration, based on the labeled amount of betaxolol hydrochloride per Tablet, of about 0.1 mg per mL when diluted. Add an amount of 0.1 N hydrochloric acid equal to about 70% of the volume of the flask, shake by mechanical means until dissolved, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter the mixture, discarding the first 20 mL of the filtrate. Concomitantly determine the absorbances of the clear filtrate and of a Standard solution of USP Betaxolol Hydrochloride RS in 0.1 N hydrochloric acid having a known concentration of about 0.1 mg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 274 nm, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) in the Tablet taken by the formula:

$$(CV)(A_U/A_S),$$

in which C, is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the Standard solution; V is the volume, in mL, of 0.1 N hydrochloric acid used to dissolve the Tablet; and A_U and A_S are the absorbances of the solution from the Tablet and the Standard solution, respectively.

Assay—

Mobile phase—Prepare a filtered mixture of 0.025 M pH 6.0 ammonium phosphate buffer, acetonitrile, and methanol (35 : 35 : 30). Mix, and degas under vacuum while stirring. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Prepare a mixture of acetonitrile and water (1 : 1).

Standard preparation—Dissolve an accurately weighed quantity of USP Betaxolol Hydrochloride RS in *Diluent* to obtain a solution having a known concentration of about 2 mg per mL.

Assay preparation—Dissolve not fewer than 20 Tablets in an appropriate accurately measured volume of *Diluent* so that the final concentration, based on the labeled amount per Tablet, is about 2 mg of betaxolol hydrochloride per mL. Sonicate until the Tablets are disintegrated. Cool to room temperature, dilute with *Diluent* to volume, mix, and filter. Use the clear filtrate as the *Assay preparation.*

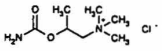
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* record the chromatogram, and measure the peak response as directed for *Procedure:* the tailing factor is not more than 3.0, 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*, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betaxolol hydrochloride ($C_{18}H_{29}NO_3 \cdot HCl$) in each Tablet taken by the formula:

$$(CV/N)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the *Standard preparation;* V is the volume of *Diluent* used to dissolve the Tablets; N is the number of Tablets taken; and r_U and r_S are the betaxolol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Bethanechol Chloride



$C_7H_{17}ClN_2O_2$ 196.67

1-Propanaminium, 2-(aminocarbonyl)oxy-*N*,*N*,*N*-trimethyl-, chloride, (±)-.

(±)-(2-Hydroxypropyl)trimethylammonium chloride carbamate [*590-63-6*].

» Bethanechol Chloride contains not less than 98.0 percent and not more than 101.5 percent of $C_7H_{17}ClN_2O_2$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Bethanechol Chloride RS.*

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: Dissolve about 50 mg in 2 mL of water, add 0.1 mL of cobaltous chloride solution (1 in 100), then add 0.1 mL of potassium ferrocyanide TS: an emerald-green color is produced, and almost entirely fades in 5 to 10 minutes (*distinction from choline chloride, which gives the same reaction but the color does not fade*).

USP 28

C: To 1 mL of a
brown precipitate is
green color.

D: A solution o

pH ⟨791⟩: betwee

Loss on drying ⟨73
than 1.0% of its wei

Residue on ignition

Heavy metals, *Meth*
add 2 mL of 1 N acet
is 0.003%.

Organic volatile im
ments.

Chloride content—
accurately weighed,
nitrate VS, then add
shake for a few min
and titrate the excess
VS. Each mL of 0.1
the content of Cl is

Related compounds
Buffer solution—T
1000-mL volumetric
volume.
Mobile phase—Pr
solution and acetonit
System Suitability ur
Standard solution
USP Bethanechol
quantitatively, and
obtain a solution hav
Bethanechol Chlorid
Test solution—Tra
accurately weighed,
dilute with *Mobile p*
System suitability
Chloride, accurately
mL of 0.1 N sodiun
minutes. Add 10 m
dilute with *Mobile p*
Chromatographic
liquid chromatograp
a 3.9- × 150-mm c
about 1.0 mL per mi
maintained at 35° an
suitability solution,
Procedure: the rela
xypropyltrimethyl ar
resolution, *R,* betwe
ride and bethanech
Standard solution, a
Procedure: the relati
not more than 10.0%
Procedure—Separ
Mobile phase, the *St*
chromatograph, reco
responses for all the
in the portion of Bet

in which *C* is the co
Chloride RS in the *St*
and is equal to 0.79 f
for any other impurit
Test solution; r_S is th
RS in the *Standar*
Bethanechol Chlorid
than 1.0% of 2-hyd
more than 0.1% of ar
impurities is not mor

Assay—
Buffer solution—T
1000-mL volumetric
µL of nitric acid to
volume. Pass throug

solution of hexanitrodiphenylamine in 0.1 N sodium hydroxide. Mix, add 15 mL of methylene chloride to each separator, shake for 1 minute, and allow the layers to separate: a deep amber color is produced in the methylene chloride layer obtained from the test solution.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.0 and 7.5.

Assay—

Hypochlorite reagent and *Standard preparation*—Prepare as directed in the *Assay* under *Carbachol Ophthalmic Solution*.

Assay preparation—Dilute, if necessary, an accurately measured volume of Intraocular Solution quantitatively and stepwise with water to obtain a solution containing about 100 µg of carbachol per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Carbachol Ophthalmic Solution*.

Carbachol Ophthalmic Solution

» Carbachol Ophthalmic Solution is a sterile solution of Carbachol in an isotonic, aqueous medium. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_6H_{15}ClN_2O_2$. It may contain suitable preservatives and antimicrobial agents.

Packaging and storage—Preserve in tight containers.

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

Identification—

A: When diluted to a concentration of about 1 mg of carbachol per mL, it responds to *Identification* test *A* under *Carbachol*.

B: Evaporate a volume of Ophthalmic Solution, equivalent to about 500 mg of carbachol, on a steam bath to dryness: the residue responds to *Identification* test *B* under *Carbachol*.

C: Evaporate a volume of Ophthalmic Solution, equivalent to about 100 mg of carbachol, on a steam bath to dryness: the residue responds to *Identification* test *D* under *Carbachol*.

Sterility ⟨71⟩: meets the requirements.

pH ⟨791⟩: between 5.0 and 7.0.

Assay—

Hypochlorite reagent—Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, allow to stand for 30 minutes, then mix equal volumes of the resulting solution and 1 N sodium hydroxide. Prepare fresh daily.

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

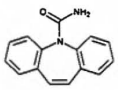
Assay preparation—Dilute an accurately measured volume of Ophthalmic Solution quantitatively and stepwise with water to obtain a solution containing about 100 µg of carbachol per mL.

Procedure—Transfer 2.0-mL portions each of the *Assay preparation* and the *Standard preparation*, and of water to provide a blank, to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid, and mix. Treat each as follows. Add 4.0 mL of *Hypochlorite reagent*, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 minutes, accurately timed. Add 2.0 mL of phenol solution (1 in 200), rinsing the walls of the flask with the solution and with additional small portions of water. Mix, and allow to stand for 5 minutes. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to assure complete acidification of all contents, then mix. Add 1.0 mL of potassium iodide solution (3 in 1000), mix, and allow to stand for 5 minutes. Add 3.0 mL of starch TS, mix, transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 590 nm, with a suitable spectrophotometer, against the blank. Calculate the quantity, in mg, of $C_6H_{15}ClN_2O_2$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.001CD(A_U/A_S),$$

in which C is the concentration, in µg per mL, of USP Carbachol RS in the *Standard preparation*, D is the dilution factor used in the *Assay preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

Carbamazepine



$C_{15}H_{12}N_2O$ 236.27

5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide.

5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide [*298-46-4*].

» Carbamazepine contains not less than 98.0 percent and not more than 102.0 percent of $C_{15}H_{12}N_2O$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

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

Identification, *Infrared Absorption* ⟨197M⟩.

X-ray diffraction ⟨941⟩—The X-ray diffraction pattern conforms to that of USP Carbamazepine RS, similarly determined.

Acidity—Add 2.0 g to 40.0 mL of water, mix for 15 minutes, and filter through paper. To a 10.0-mL aliquot of the solution so obtained add 1 drop of phenolphthalein TS, and titrate with 0.01 N sodium hydroxide VS from a 10-mL buret. Perform a blank determination, and make any necessary correction. Not more than 1.0 mL of 0.010 N sodium hydroxide is required for each 1.0 g of Carbamazepine.

Alkalinity—To a 10.0-mL aliquot of the solution prepared in the test for *Acidity* add 1 drop of methyl red TS, and titrate with 0.01 N hydrochloric acid VS from a 10-mL buret. Perform a blank determination, and make any necessary correction. Not more than 1.0 mL of 0.010 N hydrochloric acid is required for each 1.0 g of Carbamazepine.

Loss on drying ⟨731⟩—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%, a 2.0-g test specimen being used.

Chloride ⟨221⟩—Boil 1.0 g with 20.0 mL of water for 10 minutes, cool, again adjust the volume, and filter: a 10.0-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

Heavy metals, *Method II* ⟨231⟩: 0.001%.

Chromatographic purity—

Mobile phase and *System suitability solution*—Proceed as directed in the *Assay*.

Standard solution—Dissolve accurately weighed quantities of USP Carbamazepine RS, 10,11-dihydrocarbamazepine, and iminostilbene in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 0.02 mg per mL of each component. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a mixture of methanol and water (50 : 50) to volume, and mix.

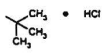
Test solution—Transfer about 100 mg of Carbamazepine, accurately weighed, to a 50-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, add about 20 mL of water, and shake. Allow the mixture to cool to room temperature, and dilute with water to volume.

Calculate the quantity, in mg, of
$O_4 \cdot \frac{1}{2}H_2O$) in the portion of

$00C)(r_U/r_S)$,

molecular weights of codeine
ous codeine phosphate, respec-
ng per mL, of USP Codeine
ation, and r_U and r_S are the peak
peak obtained from the *Assay*
ation, respectively.

de



ylethyl)amino]-2-hydroxypro-
hloride.
poxy]-3,4-dihydrocarbostyril
1-6].

ıtains not less than 98.0
an 101.5 percent of
ı the dried basis.

well-closed containers.
ᵖ *Carteolol Hydrochloride RS.*

—

o the tests for *Chloride* ⟨191⟩.
solution (1 in 100).
for 3 hours: it loses not more

: than 0.1%.
more than 0.002%.

solution of USP Carteolol
ing 0.5 mg per mL.
L of *Standard solution A* to a
ethanol to volume, and mix.
L of *Standard solution B* to a
ethanol to volume, and mix.
rteolol Hydrochloride to a 10-
ol, using heat or sonication if
with methanol to volume, and

rtions of the *Test solution* and
rting line of a thin-layer
graphy ⟨621⟩) coated with a
a gel mixture. Allow the spots
with filter paper, and saturate
ıg of a mixture of chloroform,
50 : 20 : 1). Place the plate in
ogram until the solvent front
ıgth of the plate. Remove the
-dry. Examine the plate under
e of the principal spot in the
olution corresponds to that in
ard solution A. Compare the
than the principal spot in the
solution with those of the

principal spots in the chromatograms obtained from the *Standard solutions:* no spot exceeds in size or intensity the principal spot in the chromatogram obtained from *Standard solution B* (0.2%), and the sum of all the impurity spots does not exceed 0.5%.

Assay—

pH 6.0 buffer—Dissolve 1.34 g of dibasic sodium phosphate in about 1900 mL of water, adjust with 1 M phosphoric acid to a pH of 6.0 ± 0.05, dilute with water to 2000 mL and mix.

Mobile phase—Prepare a mixture of *pH 6.0 buffer* and acetonitrile (750 : 250). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Increasing the proportion of *pH 6.0 buffer* increases resolution.]

Diluent—Prepare a mixture of *pH 6.0 buffer* and methanol (1 : 1).

Standard preparation—Dissolve an accurately weighed quantity of USP Carteolol Hydrochloride RS quantitatively in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 10.0 mL of this stock solution to a 100-mL volumetric flask containing 5 mL of acetonitrile, dilute with water to volume, and mix. This solution contains about 0.1 mg of USP Carteolol Hydrochloride RS per mL.

Resolution solution—Transfer about 50 mg of *p*-acetotoluidide to a 100-mL volumetric flask, add 50 mL of acetonitrile, and swirl to dissolve. Dilute with water to volume, and mix. Transfer 10 mL of this solution and 10 mL of the stock solution used to prepare the *Standard preparation* to a second 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains about 0.05 mg of *p*-acetotoluidide and 0.1 mg of USP Carteolol Hydrochloride RS.

Assay preparation—Transfer about 100 mg of Carteolol Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask containing 5 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 252-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure:* the relative retention times are about 0.8 for carteolol and 1.0 for *p*-acetotoluidide, and the resolution, *R*, between the carteolol peak and the *p*-acetotoluidide peak is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* 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 $C_{16}H_{24}N_2O_3 \cdot HCl$ in the portion of Carteolol Hydrochloride taken by the formula:

$$1000C(r_U/r_S),$$

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

Carteolol Hydrochloride Ophthalmic Solution

» Carteolol Hydrochloride Ophthalmic Solution is a sterile, aqueous, isotonic solution of Carteolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{24}N_2O_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

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

Identification—

A: Prepare a test solution by diluting a suitable volume of Ophthalmic Solution with water, if necessary, to obtain a solution containing about 1 mg of carteolol hydrochloride per mL. Separately apply 10 µL of the test solution and 10 µL of a Standard solution of USP Carteolol Hydrochloride RS in water containing about 1 mg per mL to the starting line of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry. Line a chromatographic chamber with filter paper, and saturate the paper with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (50 : 20 : 1). Place the plate in the chamber, 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, and allow to air-dry. Examine the plate under short-wavelength UV light: the R_F value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the Standard solution.

B: The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* 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 8.0.

Assay—

pH 6.0 buffer, *Mobile phase*, *Diluent*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Carteolol Hydrochloride.*

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Filter a portion of this solution through a filter having a porosity of 0.5 µm or finer, discarding the first 2 mL of the filtrate, and use the filtrate as the *Assay preparation.*

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 $C_{16}H_{24}N_2O_3 \cdot HCl$ in each mL of the Ophthalmic Solution taken by the formula:

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

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

Carteolol Hydrochloride Tablets

» Carteolol Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{16}H_{24}N_2O_3 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—*USP Carteolol Hydrochloride RS. USP Dehydrocarteolol Hydrochloride RS.*

Identification—The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* 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: 30 minutes.

Mobile phase—Dissolve 2.0 g of monobasic potassium phosphate in water to make 1000 mL of solution. Prepare a mixture of this solution and acetonitrile (600 : 400). Degas and filter through a filter having a porosity of 0.5 µm or finer. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Prepare a solution of USP Carteolol Hydrochloride RS in water having a known concentration of about 1.1*L* µg per mL, *L* being the labeled amount, in mg, of carteolol hydrochloride per Tablet.

USP 28

Assay—

pH 3.6 buffer, pH 7.0 buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefazolin.*

Assay preparation—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefazolin, to a 50-mL volumetric flask, dilute with *pH 7.0 buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 buffer* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Cefazolin.* Calculate the quantity, in mg, of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in each mL of the Injection taken by the formula:

$$(1000C/V)(R_U/R_S),$$

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

Cefazolin for Injection

» Cefazolin for Injection contains an amount of Cefazolin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$).

Packaging and storage—Preserve in *Containers for Injections* as described under *Injections* ⟨1⟩.

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

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—
Solution: 20 µg per mL.
Medium: 0.1 M sodium bicarbonate.

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

C: It meets the requirements of the tests for *Sodium* ⟨191⟩.

Specific rotation ⟨781S⟩: between –10° and –24°.
Test solution: 55 mg per mL, in 0.1 M sodium bicarbonate.

Bacterial endotoxins ⟨85⟩—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

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

Uniformity of dosage units ⟨905⟩: meets the requirements.
Procedure for content uniformity—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

pH ⟨791⟩: between 4.0 and 6.0, in a solution containing 100 mg of cefazolin per mL.

Water, *Method I* ⟨921⟩: not more than 6.0%.

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

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

Assay—

pH 3.6 buffer, pH 7.0 buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefazolin.*

Assay preparation 1 (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 buffer* to volume, and mix.

Assay preparation 2 (where the label states the quantity of cefazolin in a given volume of constituted solution)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 buffer* to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Cefazolin.* Calculate the quantity, in mg, of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in the container, and in the volume of constituted solution taken by the formula:

$$(CL/D)(R_U/R_S),$$

in which L is the labeled quantity, in mg, of cefazolin in the container, or in the volume of constituted solution taken; D is the concentration, in mg per mL, of cefazolin in the stock solution used in preparing *Assay preparation 1* or *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the volume of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay* value.

Cefazolin Ophthalmic Solution

» Cefazolin Ophthalmic Solution contains an amount of Cefazolin Sodium equivalent to not less than 29.7 mg and not more than 36.3 mg of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in 10.0 mL of Ophthalmic Solution. Use Cefazolin Sodium or Cefazolin for Injection that contains the designated amount of cefazolin, and prepare the Ophthalmic Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩):

Cefazolin Sodium	35 mg
Thimerosal	0.2 mg
Sodium Chloride Injection (0.9%), a sufficient quantity to make	10.0 mL

Dissolve accurately weighed quantities of Cefazolin Sodium and Thimerosal in Sodium Chloride Injection (0.9%), and dilute quantitatively, and stepwise if necessary, with Sodium Chloride Injection (0.9%) to obtain a solution containing, in each mL, 3.5 mg of Cefazolin Sodium and 0.02 mg of Thimerosal. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution. If Cefazolin for Injection is used, prepare the Ophthalmic Solution as follows. Dissolve an accurately weighed quantity of Thimerosal in Sodium Chloride Injection (0.9%), and dilute quantitatively, and stepwise if necessary, with Sodium Chloride Injection (0.9%) to obtain a solution containing 0.3 mg of Thimerosal per mL. Add 9.8 mL of the resulting solution to a vial of Cefazolin for Injection, containing 500 mg of cefazolin, and mix to obtain a stock solution. Transfer 3.3 mL of the stock solution to a 50-mL volumetric flask, dilute with Sodium Chloride Injection (0.9%) to volume, and mix. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution.

Packaging and sto
containers. Store in a
Labeling—Label it t
not to be used if a pr
USP Reference stan
Sterility—See *Steril*
sterile Preparations
pH ⟨791⟩: between
Beyond-use date:
compounded.
Compliance assay—
pH 3.6 buffer—T
sodium phosphate an
1-liter volumetric flas
and mix.
pH 7.0 buffer—Tra
phosphate and about
liter volumetric flask,
mix.
Solution A—Comb
acetonitrile in a suitab
a filter having a 5-µn
Solution B—Comb
acetonitrile in a suitab
a filter having a 5-µn
Mobile phase—Us
as directed for *Ch*
necessary (see *Systen*
Standard preparat
dissolve an accurate
7.0 buffer, and dilute
pH 7.0 buffer to obta
about 0.32 mg per m
Assay preparation
10-mL low-actinic v
volume, and mix. M
Chromatographic
liquid chromatograp
3.9-mm × 30-cm co
rate is about 2 mL
maintained at 25°. T

Time (minutes)	S
0	
0–15	
15–25	

Chromatograph the
responses as directed
than 1500 theoretical
and the relative stand
than 2.0%.
Procedure—Separ
Standard preparatio
ograph, record the ch
major peaks. Calc
($C_{14}H_{14}N_8O_4S_3$) in 1
formula:

in which C is the con
in the *Standard prep*
obtained from the *As*
respectively.

USP 28

this solution to a 100-mL
l standard solution, dilute

el states the quantity of
uted solution)—Constitute
vater, accurately measured,
specified in the labeling.
of the constituted solution
a stock solution containing
5.0 mL of this solution to a
Internal standard solution,
mix.
ie *Assay* under *Cefazolin*.
olin ($C_{14}H_{14}N_8O_4S_3$) in the
ated solution taken by the

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f cefazolin in the container,
ken; *D* is the concentration,
solution used in preparing
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dure for content uniformity,
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lution

contains an amount of
not less than 29.7 mg
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in, and prepare the
(see *Pharmaceutical*
ations ⟨795⟩):

. . . .	35 mg
. . . .	0.2 mg
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each mL, 3.5 mg of
f Thimerosal. Filter a
solution to produce a
ution. If Cefazolin for
ophthalmic Solution as
weighed quantity of
Injection (0.9%), and
se if necessary, with
) to obtain a solution
per mL. Add 9.8 mL of
Cefazolin for Injection,
and mix to obtain a
the stock solution to a
with Sodium Chloride
mix. Filter a 10.0-mL
o produce a clear and

Packaging and storage—Preserve in tight, sterile ophthalmic containers. Store in a refrigerator.

Labeling—Label it to state that it is intended for use in the eye and is not to be used if a precipitate is present.

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

Sterility—See *Sterility* under *Pharmaceutical Compounding—Non-sterile Preparations* ⟨795⟩.

pH ⟨791⟩: between 4.5 and 6.0.

Beyond-use date: 5 days after the date on which it was compounded.

Compliance assay—

pH 3.6 buffer—Transfer about 0.900 g of anhydrous dibasic sodium phosphate and about 1.298 g of citric acid monohydrate to a 1-liter volumetric flask, dissolve in and dilute with water to volume, and mix.

pH 7.0 buffer—Transfer about 5.68 g of anhydrous dibasic sodium phosphate and about 3.63 g of monobasic potassium phosphate to a 1-liter volumetric flask, dissolve in and dilute with water to volume, and mix.

Solution A—Combine 900 mL of *pH 3.6 buffer* and 100 mL of acetonitrile in a suitable container. Pass the resulting solution through a filter having a 5-µm or finer porosity, and degas.

Solution B—Combine 200 mL of *pH 3.6 buffer* and 800 mL of acetonitrile in a suitable container. Pass the resulting solution through a filter having a 5-µm or finer porosity, and degas.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Using low-actinic volumetric glassware, dissolve an accurately weighed quantity of USP Cefazolin RS in *pH 7.0 buffer*, and dilute quantitatively, and stepwise if necessary, with *pH 7.0 buffer* to obtain a solution having a known concentration of about 0.32 mg per mL. Maintain at 4° prior to injection.

Assay preparation—Transfer 1.0 mL of Ophthalmic Solution to a 10-mL low-actinic volumetric flask, dilute with *pH 7.0 buffer* to volume, and mix. Maintain at 4° prior to injection.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 3.9-mm × 30-cm column that contains 10-µm packing L1. The flow rate is about 2 mL per minute and the column temperature is maintained at 25°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–15	100→0	0→100	linear gradient
15–25	100	0	isocratic

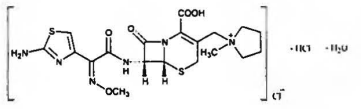
Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

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 cefazolin ($C_{14}H_{14}N_8O_4S_3$) in 10 mL of Ophthalmic Solution taken by the formula:

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

in which C is the concentration, in mg per mL, of USP Cefazolin 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.

Cefepime Hydrochloride



$C_{19}H_{25}ClN_6O_5S_2 \cdot HCl \cdot H_2O$ 571.50

Pyrrolidinium, 1-[[7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]-amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-, chloride, monohydrochloride, monohydrate, [6*R*-[6α,7β(*Z*)]]-.

1-[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7²-(*Z*)-(*O*-methyloxime), monohydrochloride, monohydrate [*123171-59-5*].

» Cefepime Hydrochloride contains the equivalent of not less than 825 µg and not more than 911 µg of cefepime ($C_{19}H_{24}N_6O_5S_2$) per mg, calculated on the anhydrous basis.

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

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 Cefepime Hydrochloride RS. USP Cefepime Hydrochloride System Suitability RS. USP Endotoxin RS.*

Identification, *Infrared Absorption* ⟨197M⟩.

Test specimen—Proceed as directed in the chapter, but do not dry.

Crystallinity ⟨695⟩: meets the requirements.

Bacterial endotoxins ⟨85⟩—Where the label states that Cefepime Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.04 USP Endotoxin Unit per mg of cefepime hydrochloride.

Water, *Method I* ⟨921⟩: between 3.0% and 4.5%.

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

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

Limit of *N*-methylpyrrolidine—

Mobile phase—Prepare a filtered and degassed mixture of 0.01 N nitric acid and acetonitrile (100 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer about 0.16 mL of *N*-methylpyrrolidine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with 0.01 N nitric acid to volume, and mix. This solution contains about 0.05 mg of *N*-methylpyrrolidine per mL.

Test solution—Transfer about 100 mg of Cefepime Hydrochloride, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with 0.01 N nitric acid to volume, and mix. [NOTE—Use this solution within 30 minutes.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a conductivity detector and a 4.6-mm × 5-cm column that contains 5-µm packing L52. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the retention time of *N*-methylpyrrolidine is not less than 8 minutes, and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for *N*-

suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chloramphenicol RS in the same *Medium*.

Tolerances—Not less than 85% *(Q)* of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$ is dissolved in 30 minutes.

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

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

Standard preparation—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer an accurately counted number of Chloramphenicol Capsules, equivalent to about 2500 mg of chloramphenicol, to a 1000-mL volumetric flask, add 100 mL of water, and heat on a steam bath until the Capsules have disintegrated. Add 300 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each Capsule taken by the formula:

$$20(C/N)(r_U/r_S),$$

in which N is the number of Capsules taken, and the other terms are as defined therein.

Chloramphenicol Cream

» Chloramphenicol Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

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

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

Identification—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.*

Minimum fill ⟨755⟩: meets the requirements.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

Standard preparation—Transfer about 40 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer an accurately weighed quantity of Chloramphenicol Cream, equivalent to about 40 mg of chloramphenicol, to a 100-mL volumetric flask, add about 80 mL of methanol, and sonicate for about 10 minutes. Cool to room temperature, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of Cream taken by the formula:

$$0.5C(r_U/r_S),$$

in which the terms are as defined therein.

Chloramphenicol Injection

» Chloramphenicol Injection is a sterile solution of Chloramphenicol in one or more suitable solvents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$. It may contain suitable buffers.

Packaging and storage—Preserve in single-dose or in multiple-dose containers.

Labeling—Label it to indicate that it is for veterinary use only.

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

Identification—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.*

Bacterial endotoxins ⟨85⟩—It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 1 mL from each container being transferred directly to the membrane filter.

pH ⟨791⟩: between 5.0 and 8.0, in a solution diluted with water (1 : 1).

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

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Chloramphenicol.*

Assay preparation—Transfer an accurately measured volume of Chloramphenicol Injection, equivalent to about 200 mg of chloramphenicol, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a 0.5-µm or finer porosity filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each mL of the Injection taken by the formula:

$$2.5(C/V)(r_U/r_S),$$

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

Chloramphenicol Ophthalmic Ointment

» Chloramphenicol Ophthalmic Ointment contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes.

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

Identification—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.

Minimum fill ⟨755⟩: meets the requirements.

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

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol.*

Standard preparation—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 25-mL volumetric

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flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 25 mg of chloramphenicol, to a suitable conical flask, add 20 mL of cyclohexane, mix, and sonicate for about 2 minutes. Add 60 mL of methanol, and mix. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume, and mix. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol. Transfer 10.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of Ophthalmic Ointment taken by the formula:

$$0.25C(r_U / r_S),$$

in which the terms are as defined therein.

Chloramphenicol Ophthalmic Solution

» Chloramphenicol Ophthalmic Solution is a sterile solution of Chloramphenicol. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

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

Labeling—The labeling states that there is a 21-day beyond-use period after dispensing.

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

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

pH ⟨791⟩: between 7.0 and 7.5, except that in the case of Ophthalmic Solution that is unbuffered or is labeled for veterinary use it is between 3.0 and 6.0.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C / V)(r_U / r_S),$$

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

Chloramphenicol for Ophthalmic Solution

» Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$, when constituted as directed.

Packaging and storage—Preserve in tight containers.

Labeling—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.

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

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

pH ⟨791⟩: between 7.1 and 7.5, in an aqueous solution containing 5 mg of chloramphenicol per mL.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

Standard preparation—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

Assay preparation—Transfer the contents of 1 container of Chloramphenicol for Ophthalmic Solution to a suitable container with the aid of *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 100 µg of chloramphenicol per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of $C_{11}H_{12}Cl_2N_2O_5$ in the container of Chloramphenicol for Ophthalmic Solution taken by the formula:

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

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

Chloramphenicol Oral Solution

» Chloramphenicol Oral Solution is a solution of Chloramphenicol in a suitable solvent. It contains one or more suitable buffers and preservatives. It has a potency of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of $C_{11}H_{12}Cl_2N_2O_5$.

Packaging and storage—Preserve in tight containers.

Labeling—Label it to indicate that it is for veterinary use only and that it is not to be used in animals raised for food production.

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

Identification—A volume of Oral Solution, equivalent to about 250 mg of chloramphenicol, responds to the *Identification* test under *Chloramphenicol Capsules*.

pH ⟨791⟩: between 5.0 and 8.5, when diluted with an equal volume of water.

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Resolution solution—Dissolve a quantity of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard preparation* to obtain a solution having a concentration of about 0.25 mg per mL.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 25 mg of ciprofloxacin, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

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 area responses for the major peaks. Calculate the quantity, in mg, of ciprofloxacin in each mL of the Injection taken by the formula:

$$(331.35/367.81)(100C/V)(r_U/r_S),$$

in which 331.35 and 367.81 are the molecular weights of ciprofloxacin and anhydrous ciprofloxacin hydrochloride, respectively; C is the concentration, in mg per mL, of USP Ciprofloxacin Hydrochloride RS in the *Standard preparation*, calculated on the anhydrous basis; V is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and r_U and r_S are the ciprofloxacin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ciprofloxacin Ophthalmic Ointment

» Ciprofloxacin Ophthalmic Ointment contains an amount of Ciprofloxacin Hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

Packaging and storage—Preserve in collapsible ophthalmic ointment tubes. Store at a temperature between 2° and 25°.

USP Reference standards ⟨11⟩—*USP Ciprofloxacin Ethylenediamine Analog RS. USP Ciprofloxacin Hydrochloride RS.*

Identification—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⟩—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.

Metal particles ⟨751⟩: meets the requirements.

Assay—

Mobile phase—Prepare a 0.005 M tetrabutylammonium phosphate solution, and adjust with phosphoric acid to a pH of 2.0. Prepare a filtered and degassed mixture of this solution and methanol (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.033 mg per mL.

Resolution solution—Dissolve an accurately weighed quantity of USP Ciprofloxacin Ethylenediamine Analog RS in a portion of the *Standard preparation* to obtain a solution containing about 0.005 mg per mL.

Assay preparation—Transfer an accurately weighed quantity of Ophthalmic Ointment, equivalent to about 750 µg of ciprofloxacin, to a screw-capped tube. Add 15 mL of solvent hexane, and shake vigorously until the Ophthalmic Ointment is dispersed. Loosen the cap, and heat in a water bath at 60° for 30 minutes, with occasional swirling. Remove from the bath, tighten the cap, and shake for 1.5 minutes while still hot. Add 25.0 mL of 0.1 N hydrochloric acid, and shake vigorously for 1.5 minutes. Allow the layers to separate, and use the lower, aqueous layer.

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 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.8 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin; and the resolution, R, between ciprofloxacin ethylenediamine analog and ciprofloxacin is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the column efficiency is not less than 500 theoretical plates; the tailing factor is not less than 0.9 and 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 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the ciprofloxacin peaks. Calculate the quantity, in mg, of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in each g of the Ophthalmic Ointment taken by the formula:

$$(331.34/385.82)(25C/W)(r_U/r_S),$$

in which 331.34 and 385.82 are the molecular weights of ciprofloxacin and ciprofloxacin hydrochloride monohydrate, respectively; C is the concentration, in mg per mL, of USP Ciprofloxacin Hydrochloride RS in the *Standard preparation;* W is the weight, in g, of Ophthalmic Ointment taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Ciprofloxacin Ophthalmic Solution

» Ciprofloxacin Ophthalmic Solution is a sterile, aqueous solution of Ciprofloxacin Hydrochloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

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

USP Reference standards ⟨11⟩—*USP Ciprofloxacin Hydrochloride RS. USP Ciprofloxacin Ethylenediamine Analog RS.*

Identification—Dilute a volume of Ophthalmic Solution with water to obtain a test solution containing about 3 mg of ciprofloxacin per mL. Prepare a Standard solution of USP Ciprofloxacin Hydrochloride RS in water containing about 3.5 mg per mL. Proceed as directed in *Identification* test *B* under *Ciprofloxacin Hydrochloride*, beginning with "Separately apply, as 1-cm bands," except to use 3 µL each of the test solution and the Standard solution instead of 5 µL. The specified result is obtained.

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 5.5.

Assay—

Mobile phase—Prepare 0.005 M tetrabutylammonium phosphate, and adjust with phosphoric acid to a pH of 2.0. Prepare a degassed and filtered mixture of this solution and methanol (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Ciprofloxacin Hydrochloride RS in water to obtain a solution having a known concentration of about 0.14 mg per mL.

Resolution solution—Dissolve a quantity of USP Ciprofloxacin Ethylenediamine Analog RS in a portion of the *Standard preparation* to obtain a solution containing about 0.01 mg per mL.

Assay preparation—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 6 mg of ciprofloxacin, to a 50-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 4.6-mm × 25-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed under *Procedure:* the relative retention times are about 0.8 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin, and the resolution, R, between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak is not less than 1.5. Chromatograph the *Standard preparation*,