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 base-deactivated packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	80	20	equilibration
0–5	80	20	isocratic
5–15	80→40	20→60	linear gradient
15–25	40	60	isocratic
25–30	40→0	60→100	linear gradient

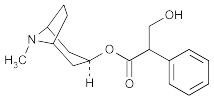
Chromatograph replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.8 for the atracurium besylate *trans-trans*-isomer, 0.9 for the *cis-trans*-isomer, and 1.0 for the *cis-cis*-isomer; the resolution, *R,* between the atracurium besylate *trans-trans*-isomer and the *cis-trans*-isomer and between the atracurium besylate *cis-trans*-isomer and the *cis-cis*-isomer is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the three atracurium besylate isomer peaks. Calculate the quantity, in mg, of atracurium besylate ($C_{65}H_{82}N_2O_{18}S_2$) in each mL of the Injection taken by the formula:

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

in which *C* is the concentration, in mg per mL, of USP Atracurium Besylate RS in the *Standard preparation; V* is the volume, in mL, of Injection taken for the *Assay preparation;* and r_U and r_S are the sums of the peak responses of the atracurium besylate *trans-trans, trans-cis,* and *cis-cis*-isomers obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Atropine



$C_{17}H_{23}NO_3$ 289.37
Benzeneacetic acid, α-(hydroxymethyl)-8-methyl-8-azabicyclo [3.2.1]oct-3-yl ester, *endo*-(±)-.
1α*H*,5α*H*-Tropan-3α-ol (±)-tropate (ester) [51-55-8].

» Atropine contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{17}H_{23}NO_3$, calculated on the anhydrous basis.

Caution—Handle Atropine with exceptional care, since it is highly potent.

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

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

Identification—

A: Dissolve 30 mg of Atropine and 36 mg of USP Atropine Sulfate RS in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 minute, allow the layers to separate, and filter the chloroform extracts through separate filters of about 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide: the IR absorption spectrum, determined in a 1-mm cell, of the solution obtained from the test specimen exhibits maxima only at the same wavelengths as that of the solution obtained from the Reference Standard.

B: To a 1 in 50 solution in 3 N hydrochloric acid add gold chloride TS: a lusterless precipitate is formed (*distinction from hyoscyamine, which, similarly treated, yields a lustrous precipitate*).

Melting range ⟨741⟩: between 114° and 118°.

Angular rotation ⟨781A⟩: the angular rotation of this solution, a 200-mm tube being used, is between –0.70° and +0.05° (limit of hyoscyamine).

Test solution—Dissolve 1 g, previously dried at 105° for 1 hour, in sufficient 50% alcohol (w/w) to obtain a volume of 20 mL at 25°.

Water, *Method I* ⟨921⟩: not more than 0.2%.

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

Readily carbonizable substances ⟨271⟩**—**Dissolve 200 mg in 5 mL of 2 N sulfuric acid: the solution has no more color than *Matching Fluid A,* and the solution is colored no more than light yellow upon the addition of 0.2 mL of nitric acid.

Limit of foreign alkaloids and other impurities—Prepare a solution of Atropine in methanol containing 20 mg per mL, and, by quantitative dilution of a portion of this solution with methanol, prepare a second solution of Atropine containing 1 mg per mL. Apply 25 µL of the first (20 mg per mL) Atropine solution, 1 µL of the second (1 mg per mL) Atropine solution, and 5 µL of a methanol solution of USP Atropine Sulfate RS containing 24 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.5-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, acetone, and diethylamine (5:4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with potassium iodoplatinate TS: the R_F value of the principal spot obtained from each test solution corresponds to that obtained from the Reference Standard solution; no secondary spot obtained from the first Atropine solution exhibits intensity equal to or greater than the principal spot obtained from the second Atropine solution (0.2%).

Assay—Dissolve about 400 mg of Atropine, accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of $C_{17}H_{23}NO_3$.

Atropine Sulfate

$(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ 694.83
Benzeneacetic acid, α-(hydroxymethyl)-, 8-methyl-8-azabicyclo [3.2.1]oct-3-yl ester, *endo*-(±)-, sulfate (2:1) (salt), monohydrate.
1α*H*,5α*H*-Tropan-3-α-ol (±)-tropate (ester), sulfate (2:1) (salt) monohydrate [5908-99-6].
Anhydrous 676.83 [55-48-1].

» Atropine Sulfate contains not less than 98.5 percent and not more than 101.0 per cent of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$, calculated on the anhydrous basis.

Caution—Handle Atropine Sulfate with exceptional care, since it is highly potent.

Packaging and storage—Preserve in tight containers.

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

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: A solution (1 in 20) meets the requirements of the tests for *Sulfate* ⟨191⟩.

Melting temperature, *Class Ia* ⟨741⟩: not lower than 187 °, determined after drying at 120 ° for 4 hours. [NOTE—Since anhydrous Atropine Sulfate is hygroscopic, determine its melting temperature promptly on a specimen placed in the capillary tube immediately after drying.]

Angular rotation ⟨781A⟩**—**The observed rotation, in degrees, multiplied by 200, and divided by the length, in mm, of the polarimeter tube used, is between –0.60° and +0.05 ° (limit of hyoscyamine).

Test solution—Dissolve 1 g, accurately weighed, in water to make a volume of 20 mL at 25 °.

Acidity—Dissolve 1.0 g in 20 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.30 mL is required to produce a yellow color.

Water, *Method I* ⟨921⟩: not more than 4.0%.

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

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 per cent 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 per cent and not more than 110.0 per cent 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