cold water, then with 5 mL of cold alcohol, and finally with 5 mL of cold ether, and dry in vacuum over silica gel for 3 hours. The epinephrine so obtained responds to the *Identification* test under *Epinephrine*, and its specific rotation (see *Optical Rotation* ⟨781⟩), determined by dissolving 200 mg, accurately weighed, in sufficient dilute hydrochloric acid (1 in 20) to make 10.0 mL, is between –50° and –53.5°.

Melting range ⟨741⟩: between 147° and 152°, with decomposition.

Loss on drying ⟨731⟩—Dry it in vacuum over silica gel for 3 hours: it loses not more than 0.5% of its weight.

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

Limit of adrenalone—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩) at 310 nm, determined in a solution in dilute hydrochloric acid (1 in 200) containing 4 mg per mL, is not more than 0.2.

Limit of norepinephrine bitartrate—

Epinephrine standard solution—Dilute with methanol an accurately measured volume of an aqueous solution of USP Epinephrine Bitartrate RS containing about 200 mg per mL to obtain a solution having a known concentration of about 20 mg per mL.

Norepinephrine standard solution—Dilute with methanol an accurately measured volume of an aqueous solution of USP Norepinephrine Bitartrate RS containing 8.0 mg per mL to obtain a solution having a known concentration of 0.80 mg per mL.

Test solution—Dissolve 200 mg of Epinephrine Bitartrate in 1.0 mL of water, dilute with methanol to 10.0 mL, and mix.

Procedure—Apply 5-µL portions of *Epinephrine standard solution*, *Norepinephrine standard solution*, and *Test solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in an unsaturated tank using a solvent system consisting of *n*-butanol, water, and formic acid (7:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm circulating air. Spray with Folin-Ciocalteu Phenol TS, followed by sodium carbonate solution (1 to 10): the R_F value of the principal spot obtained from the *Test solution* corresponds to that obtained from the *Epinephrine standard solution*. Any spot obtained from the *Test solution* is not larger nor more intense than the spot with the same R_F value obtained from *Norepinephrine standard solution*, corresponding to not more than 4.0% of norepinephrine bitartrate.

Assay—Dissolve about 500 mg of Epinephrine Bitartrate, accurately weighed, in 20 mL of glacial acetic acid, warming slightly if necessary to effect solution. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.33 mg of $C_9H_{13}NO_3 \cdot C_4H_6O_6$.

Epinephrine Bitartrate Inhalation Aerosol

» Epinephrine Bitartrate Inhalation Aerosol is a suspension of microfine Epinephrine Bitartrate in propellants in a pressurized container. It contains *not less than 90.0 percent and not more than* 110.0 percent of the labeled amount of epinephrine bitartrate ($C_9H_{13}NO_3 \cdot C_4H_6O_6$).

Packaging and storage—Preserve in small, nonreactive, light-resistant aerosol containers equipped with metered-dose valves and provided with oral inhalation actuators.

USP Reference standards ⟨11⟩—
USP Epinephrine Bitartrate RS

Identification—

A: Place 10 mL of water in a small beaker, and deliver 3 sprays from the Aerosol under the surface of the water, actuating the valve by pressing the tip against the bottom of the beaker. Filter, and to 5 mL of the filtrate add 1 drop of dilute hydrochloric acid (1 in 120). Add 0.5 mL of 0.1 N iodine, allow to stand for 5 minutes, and add 1 mL of 0.1 N sodium thiosulfate: a red-brown color is produced.

B: Actuate the valve of the Aerosol by pressing the tip against a station of a white porcelain spot plate. Cover the spot with 2 or 3 drops of a mixture of 3 volumes of pyridine and 1 volume of acetic anhydride: an emerald-green color is produced.

Delivered dose uniformity over the entire contents: meets the requirements for *Metered-Dose Inhalers* under *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* ⟨601⟩.

PROCEDURE FOR DOSE UNIFORMITY—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in a freshly prepared sodium bisulfite solution (1 in 500), and dilute quantitatively and stepwise with the same sodium bisulfite solution as necessary to obtain a solution having a known concentration of about 15 µg per mL.

Test preparation—Discharge the minimum recommended dose into the sampling apparatus and detach the inhaler as directed. Rinse the apparatus (filter and interior) with four 5.0-mL portions of a freshly prepared sodium bisulfite solution (1 in 500), and transfer the resulting solutions quantitatively to a 50-mL centrifuge tube. Add 10 mL of chloroform, insert the stopper, shake vigorously for 1 minute and centrifuge for 5 minutes. Use the clear supernatant as directed in the *Procedure*.

Procedure—Into three separate flasks, transfer the *Test preparation*, 20.0 mL of the *Standard preparation*, and 20.0 mL of water to provide the blank. To each flask add 100 µL of *Ferro-citrate solution* and 1.0 mL of *Buffer solution*, and mix. Concomitantly determine the absorbances with a suitable spectrophotometer, in 5-cm cells, of the solutions from the *Test preparation* and the *Standard preparation*, at the wavelength of maximum absorbance at about 530 nm, against the blank. Calculate the quantity, in µg of $C_9H_{13}NO_3 \cdot C_4H_6O_6$ contained in the minimum dose taken by the formula:

$$(20CN)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*; N is the number of sprays discharged to obtain the minimum recommended dose; and A_U and A_S are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

Particle size—Proceed with Epinephrine Bitartrate Inhalation Aerosol as directed in the test for *Particle size* under *Isoproterenol Sulfate Inhalation Aerosol*. It meets the limits of the test.

Assay—

Ferro-citrate solution and *Buffer solution*—Prepare as directed under *Epinephrine Assay* ⟨391⟩.

Standard preparation—Prepare as directed under *Delivered dose uniformity over the entire contents*.

Assay preparation—[NOTE—A suitable specimen beaker is one having a small indentation formed on its inside bottom