Assay preparation—Dissolve an accurately weighed portion of Cream, equivalent to about 25 mg each of dioxybenzone and oxybenzone, in methanol in a 100-mL volumetric flask, dilute with methanol to volume, and mix. Pipet 1 mL of this solution into a 15-mL conical test tube, evaporate on a water bath just to dryness, using a gentle stream of air, and dissolve the residue in about 200 µL of methanol.

Procedure—Prepare sheets of chromatographic paper (Whatman No. 1 or equivalent), each measuring about 23 × 28.5 cm, as follows. Immerse the sheets in a 1 in 20 solution of light mineral oil in solvent hexane, withdraw them immediately, and allow to dr y in air. On one sheet mark a starting line about 2.5 cm from the long edge, and apply the entire *Assay preparation* as a uniform streak along the starting line, using a stream of air or an air blower, if necessar y, to maintain the width of the streak between 5 mm and 10 mm. Rinse the conical test tube, which contained the *Assay preparation*, with about 100 µL of methanol, and apply the rinse to the starting line. Similarly, repeat the rinsing and streaking with two additional portions of methanol, and then allow the paper to dr y in air for 5 minutes.

Staple together the short edges of the paper to form a cylinder, and place it in a 12- × 25-cm cylindrical chromatographic chamber containing about 40 mL of a mobile solvent consisting of a mixture of equal volumes of acetone and water. Seal the chamber, and allow the chromatogram to develop for 2 hours.

Remove the paper from the chamber, air-dr y, then remove the staples, and view the chromatogram under short-wavelength (254 nm) UV radiation. Mark the two bands representing the separated dioxybenzone and oxybenzone, respectively. [NOTE—Determine the relative position of each benzone on the chromatogram by applying suitable aliquots of each *Standard preparation* to another prepared chromatographic sheet, and developing the chromatogram in a manner similar to that described for the *Assay preparation*.] Cut the marked bands from the sheet, and then, keeping the band segments separate, cut each into several pieces to facilitate extraction. Place the pieces from each band in separate glass-stoppered, 50-mL conical flasks, add 20.0 mL of methanol to each flask, and shake gently for 30 minutes.

To provide the chromatographic blank, treat one of the prepared chromatographic sheets in the same manner as described above, but omit the application of the *Assay preparation*. Cut from the chromatographed paper the areas corresponding to the bands produced by the benzones from the *Assay preparation*, and in the same manner extract the blank bands for 30 minutes with 20.0 mL of methanol.

Concomitantly determine the absorbance of each of the 4 solutions thus prepared, and of each of the *Standard preparations*, in a 1-cm cell at the wavelength of maximum absorbance at about 325 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of dioxybenzone ($C_{14}H_{12}O_4$) in the portion of Cream taken by the formula:

$$2C(A_U - A_B) / A_S$$

in which C is the concentration, in µg per mL, of USP Dioxybenzone RS in the dioxybenzone *Standard preparation*, and A_U, A_B, and A_S are the absorbances of the dioxybenzone solution from the *Assay preparation*, the dioxybenzone chromatographic blank solution, and the dioxybenzone *Standard preparation*, respectively. In a similar manner, calculate the quantity, in mg, of oxybenzone ($C_{14}H_{12}O_3$) in the portion of Cream taken, using as C, A_U, A_B, and A_S the respective values pertaining to the oxybenzone determination.

Diphenhydramine Citrate

$C_{17}H_{21}NO \cdot C_6H_8O_7$ 447.48

Ethanamine, 2-(diphenylmethoxy)-*N*,*N*-dimethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1).

2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine citrate (1:1) [88637-37-0].

» Diphenhydramine Citrate contains not less than 98.0 per cent and not more than 100.5 percent of $C_{17}H_{21}NO \cdot C_6H_8O_7$, calculated on the dried basis.

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

USP Reference standards ⟨11⟩**—**
USP Diphenhydramine Citrate RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 700 µg per mL.

Medium: water.

C: It responds to the test for *Citrate* ⟨191⟩.

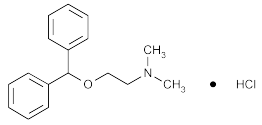
Melting range ⟨741⟩: between 146° and 150°, but the range between beginning and end of melting does not exceed 2°.

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⟩**—**To about 8 g, accurately weighed, add 5 mL of sulfuric acid, and char. After the substance is thoroughly charred, add 4 mL of nitric acid and a few drops of sulfuric acid, heat gently until fumes are no longer evolved, and ignite at 800 ± 25° until the carbon is consumed. Place in a muffle furnace at 550 ± 50° for about 1 hour. Continue the ignition until constant weight is attained: not more than 0.1% remains.

Assay—Dissolve about 1.6 g of Diphenhydramine Citrate, accurately weighed, in a mixture of 100 mL of glacial acetic acid and 20 mL of xylene. Add 20 mL of mer curic acetate TS, and titrate with 0.1 N per chloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N per chloric acid is equivalent to 44.75 mg of $C_{17}H_{21}NO \cdot C_6H_8O_7$.

Diphenhydramine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$ 291.82

Ethanamine, 2-(diphenylmethoxy)-*N*,*N*-dimethyl-, hydrochloride.

2-(Diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride [147-24-0].

» Diphenhydramine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 percent of $C_{17}H_{21}NO \cdot HCl$, calculated on the dried basis.

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

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

Identification—

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

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

C: It responds to the tests for *Chloride* ⟨191⟩.

Melting range ⟨741⟩**:** between 167° and 172°.

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 0.1%.

Assay—

Mobile phase—Prepare a solution of acetonitrile, water, and triethylamine (50:50:0.5), adjust with glacial acetic acid to a pH of 6.5, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Diphenhydramine Hydrochloride RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

Assay preparation—Transfer about 25 mg of Diphenhydramine Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with water to volume, mix, and filter.

System suitability solution—Dissolve about 5 mg of benzophenone in 5 mL of acetonitrile, dilute with water to 100 mL, and mix. Transfer 1.0 mL of this solution and 5 mg of diphenhydramine hydrochloride to a 10-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between the benzophenone and diphenhydramine peaks is not less than 2.0. Chromatograph replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the tailing factor for the diphenhydramine hydrochloride peak 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 $C_{17}H_{21}NO \cdot HCl$ in the portion of Diphenhydramine Hydrochloride taken by the formula:

$$50C(r_U / r_S)$$

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

Diphenhydramine Hydrochloride Capsules

» Diphenhydramine Hydrochloride Capsules contain not less than 90.0 per cent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO \cdot HCl$.

Packaging and storage—Preserve in tight containers.

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

Identification—

A: The contents of the Capsules meet the requirements under *Identification—Organic Nitrogenous Bases* ⟨181⟩.

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

Dissolution, *Procedure for a Pooled Sample* ⟨711⟩**—**

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Mobile phase and *Chromatographic system*—Prepare as directed in the *Assay*.

Procedure—Inject a measured volume (about 50 µL) of a filtered portion of the solution under test into the chromatograph, record the chromatogram, and measure the response for the major peak. Determine the quantity of $C_{17}H_{21}NO \cdot HCl$ dissolved in comparison with a Standard solution having a known concentration of USP Diphenhydramine Hydrochloride RS in the same medium and similarly chromatographed.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{17}H_{21}NO \cdot HCl$ is dissolved in 30 minutes.

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

Assay—

Mobile phase, Standard preparation, System suitability solution, and *Chromatographic system*—Prepare as directed in the *Assay* under *Diphenhydramine Hydrochloride*.

Assay preparation—Weigh and combine the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the combined Capsule contents, equivalent to about 50 mg of diphenhydramine hydrochloride, to a 100-mL volumetric flask. Dissolve in and dilute with water to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Diphenhydramine Hydrochloride*. Calculate the quantity, in mg, of $C_{17}H_{21}NO \cdot HCl$ in the portion of Capsule contents taken by the formula:

$$100C(r_U / r_S)$$

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

Diphenhydramine Hydrochloride Injection

»Diphenhydramine Hydrochloride Injection is a sterile solution of Diphenhydramine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{17}H_{21}NO \cdot HCl$.

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

USP Reference standards ⟨11⟩**—**
USP Diphenhydramine Hydrochloride RS
USP Endotoxin RS

Identification—

A: Dilute a volume of Injection, equivalent to about 50 mg of diphenhydramine hydrochloride, with 0.03 N sulfuric acid to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* ⟨181⟩, beginning with "Transfer the liquid to a separator": the Injection meets the requirements of the test.

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