

tion that liberates 40 µg of tyrosine per mL of test solution.

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

USP Reference standards (11)—

USP Papain RS

pH (791): between 4.8 and 6.2, in a solution (1 in 50).

Loss on drying (731)—Dry it in a vacuum oven at 60° for 4 hours: it loses not more than 7.0% of its weight.

Assay (Casein digestive power)—

Dibasic sodium phosphate, 0.05 M—Dissolve 7.1 g of anhydrous dibasic sodium phosphate in water to make 1000 mL. Add 1 drop of toluene as a preservative.

Citric acid, 0.05 M—Dissolve 10.5 g of citric acid monohydrate in water to make 1000 mL. Add 1 drop of toluene as a preservative.

Casein substrate—Disperse 1 g of Hammersten-type casein in 50 mL of 0.05 M Dibasic sodium phosphate. Place in a boiling water bath for 30 minutes with occasional stirring. Cool to room temperature, and add 0.05 M Citric acid to adjust to a pH of 6.0 ± 0.1. Stir the solution rapidly and continuously during the addition of the 0.05 M Citric acid to prevent precipitation of the casein. Dilute with water to 100 mL. Prepare fresh daily.

Buffer solution (Phosphate-Cysteine Disodium ethylenediaminetetraacetate Buffer)—Dissolve 3.55 g of anhydrous dibasic sodium phosphate in 400 mL of water in a 500-mL volumetric flask. Add 7.0 g of disodium edetate and 3.05 g of cysteine hydrochloride monohydrate. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 6.0 ± 0.1, dilute with water to volume, and mix. Prepare fresh daily.

Trichloroacetic acid solution—Dissolve 30 g of reagent grade trichloroacetic acid in water, and dilute with water to 100 mL. This solution may be stored at room temperature.

Standard preparation—Weigh accurately 100 mg of USP Papain RS in a 100-mL volumetric flask, and add Buffer solution to dissolve. Dilute with Buffer solution to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Buffer solution to volume, and mix. Use within 30 minutes after preparation.

Assay preparation—Transfer an accurately weighed amount of Papain, equivalent to about 100 mg of USP Papain RS, to a 100-mL volumetric flask, dilute with Buffer solution to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Buffer solution to volume, and mix.

Procedure—Into each of 12 test tubes (18 × 150-mm) pipet 5.0 mL of Casein substrate. Place in a water bath at 40°, and allow 10 minutes to reach bath temperature. Into each of two of the tubes (the tests are run in duplicate except for the blanks) labeled S₁, pipet 1.0 mL of the Standard preparation and 1.0 mL of the Buffer solution, mix by swirling, note zero time, insert the stopper, and replace in the bath. Into each of 2 other tubes, labeled S₂, pipet 1.5 mL of Standard preparation and 0.5 mL of Buffer solution, and proceed as before. Repeat this procedure for 2 tubes, labeled S₃, to which 2.0 mL of Standard preparation is added, and for 2 tubes, labeled U₂, to which 1.5 mL of Assay preparation and 0.5 mL of Buffer solution are added. After 60 minutes, accurately timed, add to all 12 tubes 3.0 mL of Trichloroacetic acid solution, and shake vigorously. With the 4 tubes to which no Standard preparation or Assay preparation were added, prepare blanks by pipeting, respectively, 1.0 mL of Standard preparation and 1.0 mL of Buffer solution; 1.5 mL of Standard preparation and 0.5 mL of Buffer solution; 2.0 mL of Standard preparation; and 1.5 mL of Assay preparation and 0.5 mL of Buffer solution. Replace all tubes in the 40° water bath, for 30 to 40 minutes, to allow to coagulate fully the precipitated protein. Filter through medium-porosity filter paper, discarding the first 3 mL of the filtrate (filtrates used are clear). Read the absorbances, at 280 nm, of the filtrates of all solutions against their respective blanks. Plot the absorbance readings for S₁, S₂, and S₃ against the enzyme concentration of each corre-

sponding level. By interpolation from this curve, taking into consideration dilution factors, calculate the potency in Units, in the weight of Papain taken by the formula:

$$(50,000 / 3)CA$$

in which 50,000/3 is a factor derived by the expression 100(50/2)(10/1.5), C is the concentration, in mg per mL, obtained from the standard curve, and A is the activity of the Reference Standard in Units per mg.

Papain Tablets for Topical Solution

» Papain Tablets for Topical Solution contain not less than 100.0 percent of the labeled potency.

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

USP Reference standards (11)—

USP Papain RS

Completeness of solution (641)—Prepare a solution of 50 Tablets in 500.0 mL of water, allow to stand for 4 hours, filter through 2 superimposed, matched-weight, 47-mm diameter, 0.8-µm porosity membrane filters, and wash the residue by rinsing the flask at the sides of the holder with water. Dry both filters in a desiccator under vacuum, over phosphorus pentoxide, for 6 to 18 hours, weigh the filters separately, and subtract the weight of the lower filter from that of the upper filter: the difference in the weights is not more than 50 mg (1 mg per Tablet).

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Disintegration (701): not more than 15 minutes at 23 ± 2°.

pH (791): between 6.9 and 8.0, determined in a solution of 1 Tablet in 10 mL.

Assay—

Dibasic sodium phosphate, 0.05 M; Citric acid, 0.05 M; Casein substrate; Buffer solution; Trichloroacetic acid solution; and Standard preparation—Prepare as directed in the Assay under Papain.

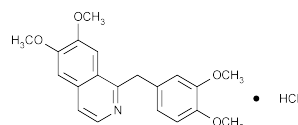
Assay preparation—Place a counted number of Papain Tablets for Topical Solution, equivalent to about 600,000 USP Units of Papain, in a 100-mL volumetric flask, dissolve in Buffer solution, dilute with Buffer solution to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with Buffer solution to volume, and mix.

Procedure—Proceed as directed for Procedure in the Assay under Papain. By interpolation from the standard curve, calculate the potency, in Units, in the number of Tablets taken by the formula:

$$(50,000 / 3)CA$$

in which the factors are as defined therein.

Papaverine Hydrochloride



C₂₀H₂₁NO₄ · HCl 375.85

Isoquinoline, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-, hydrochloride.
6,7-Dimethoxy-1-veratrylisoquinoline hydrochloride [61-25-6].

» Papaverine Hydrochloride contains not less than 98.5 percent and not more than 100.5 percent of $C_{20}H_{21}NO_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards (11)—
USP Papaverine Hydrochloride RS

Completeness of solution—A 1 in 15 solution in chloroform is clear and free from undissolved solid.

Identification—

A: *Infrared Absorption* (197K).

B: *Ultraviolet Absorption* (197U)—

Solution: 2.5 µg per mL.

Medium: 0.1 N hydrochloric acid.

Absorptivities at 251 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: A solution (1 in 50) responds to the tests for *Chloride* (191).

pH (791): between 3.0 and 4.5, in a solution (1 in 50).

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%.

Limit of cryptopine, thebaine, or other organic impurities—Dissolve 50 mg in 2 mL of sulfuric acid in a small test tube: the resulting solution is not more yellow-brown in color than *Matching Fluid S* (see *Readily Carbonizable Substances Test* (271)), and it is not more pink than a standard prepared, in equal volume, by diluting 3.0 mL of 0.1 N potassium permanganate with water to 1000 mL.

Assay—Dissolve about 700 mg of Papaverine Hydrochloride, accurately weighed, in 80 mL of glacial acetic acid; add 10 mL of mercuric acetate TS and 1 drop of crystal violet TS; and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.59 mg of $C_{20}H_{21}NO_4 \cdot HCl$.

Papaverine Hydrochloride Injection

» Papaverine Hydrochloride Injection is a sterile solution of Papaverine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

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

USP Reference standards (11)—
USP Endotoxin RS
USP Papaverine Hydrochloride RS

Identification—

A: Add 2 mL of alcohol to 1 mL of Injection, and evaporate on a steam bath, with the aid of a stream of nitrogen, to dryness. Dry the residue at 105° for 2 hours: it responds to *Identification test A* under *Papaverine Hydrochloride*.

B: It responds to *Identification test C* under *Papaverine Hydrochloride*.

Bacterial endotoxins (85)—It contains not more than 2.9 USP Endotoxin Units per mg of papaverine hydrochloride.

pH (791): not less than 3.0.

Other requirements—It meets the requirements under *Injections* (1).

Assay—Transfer 1.0 mL of Injection to a 200-mL volumetric flask, and dilute with water to volume. Pipet 3 mL of this solution into a separator, add 10 mL of water, and render alkaline with 6 N ammonium hydroxide. Extract the alkaloid with successive 5-mL portions of chloroform, and evaporate the extracts to dryness. Dissolve the residue in 0.1 N hydrochloric acid, and dilute with the same medium to 100.0 mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Papaverine Hydrochloride RS in 0.1 N hydrochloric acid having a known concentration of about 4.5 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 251 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{20}H_{21}NO_4 \cdot HCl$ in the portion of Injection taken by the formula:

$$6.67C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Papaverine Hydrochloride RS in the Standard solution, and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Papaverine Hydrochloride Tablets

» Papaverine Hydrochloride Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—
USP Papaverine Hydrochloride RS

Identification—Add a portion of powdered Tablets, equivalent to about 30 mg of papaverine hydrochloride, to 10 mL of 0.1 N hydrochloric acid in a separator. Extract the mixture with 10 mL of chloroform, filter the chloroform phase through paper, evaporate the solvent on a steam bath, and dry the residue at 105° for 2 hours: it responds to *Identification test A* under *Papaverine Hydrochloride*.

Dissolution (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{20}H_{21}NO_4 \cdot HCl$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Papaverine Hydrochloride RS in the same *Medium*.

Tolerances—Not less than 80% (Q) of the labeled amount of $C_{20}H_{21}NO_4 \cdot HCl$ is dissolved in 30 minutes.

Uniformity of dosage units (905): meet the requirements.

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 250-mL volumetric flask, add 50 mL of water and 3 mL of hydrochloric acid, mix, and allow to stand for 15 minutes with occasional agitation. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with water to provide a solution containing approximately 2.4 µg of papaverine hydrochloride per mL. Concomitantly determine the absorbances of this solution and a solution