Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Trospium chloride related compound B[a]	0.7–0.8	0.15
Trospium	1.0	—
Benzilic acid (trospium chloride related compound A)	1.9–2.8	0.15
Any other individual impurity	—	0.10

[a] (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl hydroxydiphenylacetate.

- **PROCEDURE 2: LIMIT OF TROSPIUM CHLORIDE RELATED COMPOUND C**

System suitability solution: 0.5 mg/mL of USP Trospium Chloride RS and 0.5 mg/mL of USP Trospium Chloride Related Compound C RS in methanol
Standard solution: 0.1 mg/mL of USP Trospium Chloride Related Compound C RS in methanol
Sample solution: 100 mg/mL of Trospium Chloride in methanol
Chromatographic system
(See *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*.)
Mode: TLC
Plate: 0.25-mm layer of chromatographic silica gel mixture
Application volume: 10 µL
Developing distance: Two-thirds of the length of the plate
Developing solvent system: Acetonitrile, glacial acetic acid, and hydrochloric acid (45: 1: 3.5)
Spray reagent 1: Use Dragendorff's TS.
Spray reagent 2: 5 g/L of sodium nitrite in water
System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: The chromatogram shows two clearly visible and separated spots.
Analysis
Samples: *Standard solution* and *Sample solution*
Allow the spots to dry in a current of warm air until the odor of acetic acid is no longer perceptible. Spray the plate with *Spray reagent 1*, and subsequently with *Spray reagent 2*.
Acceptance criteria: Any spot from the *Sample solution* corresponding to trospium chloride related compound C is not more intense than the corresponding spot from the *Standard solution* (0.1%).

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry the sample at 105° to constant weight: it loses NMT 0.5% of its weight.
- **pH** ⟨**791**⟩

Sample solution: 10 mg/mL of Trospium Chloride in carbon dioxide-free water
Acceptance criteria: 5.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from light. Store at room temperature.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Trospium Chloride RS
USP Trospium Chloride Related Compound A RS
Benzilic acid.
$C_{14}H_{12}O_3$ 228.24 [76-93-7]
USP Trospium Chloride Related Compound B RS
Nortropane benzilate;
(1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl hydroxydiphenylacetate.
$C_{21}H_{23}NO_3$ 337.41
USP Trospium Chloride Related Compound C RS
Azoniaspironortropanol chloride;
(1*R*,3*r*,5*S*)-3-Hydroxyspiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium] chloride.
$C_{11}H_{20}ClNO$ 217.74

Crystallized Trypsin

» Crystallized Trypsin is a proteolytic enzyme crystallized from an extract of the pancreas of healthy bovine or porcine animals, or both. When assayed as directed herein, it contains not less than 2500 USP Trypsin Units in each mg, calculated on the dried basis, and not less than 90.0 percent and not more than 110.0 percent of the labeled potency.

NOTE—Determine the suitability of the substrates and check the adjustment of the spectrophotometer by performing the *Assay* using USP Crystallized Trypsin Reference Standard.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

USP Reference standards ⟨11⟩**—**
USP Trypsin Crystallized RS

Solubility test—An amount, equivalent to 500,000 USP Trypsin Units, is soluble in 10 mL of water and in 10 mL of saline TS.

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩**—**It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species.

Loss on drying ⟨731⟩**—**Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Residue on ignition ⟨281⟩: not more than 2.5%.

Limit of chymotrypsin—

0.067 M Phosphate buffer, pH 7.0—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of dibasic sodium phosphate solution. Adjust dropwise, if necessary, with dibasic sodium phosphate solution to a pH of 7.0.

Substrate solution—Dissolve 23.7 mg of N-acetyl-L-tyrosine ethyl ester, suitable for use in determining chymotrypsin, in about 50 mL of *0.067 M Phosphate buffer, pH 7.0* with warming. When cool, dilute with additional pH 7.0 buffer to 100 mL. (*Substrate solution* may be stored in the frozen state and used after thawing; it is important, however, to freeze immediately after preparation.)

Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing 650 USP Trypsin Units per mL.

Procedure—Conduct the test in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 0.1° in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance to ensure that the temperature does not change by more than 0.5°. Pipet 200 µL of 0.0010 N hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance reads 0.200 at 237 nm. Pipet 200 µL of *Crystallized Trypsin solution*

into another 1-cm cell, add 3.0 mL of the *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—This order of addition is to be followed.] At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are of less importance than the constancy of the rate of change of absorbance. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. The duplicate run at the same dilution should match the first run in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this test. Calculate the number of USP Chymotrypsin Units per mg of Crystallized Trypsin taken by the formula:

$$(A_2 - A_1) / (0.0075TW)$$

in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbance. Not more than 50 USP Chymotrypsin Units per 2500 USP Trypsin Units is found, indicating the presence of not more than approximately 5% of chymotrypsin.

Assay—

0.067 M Phosphate buffer, pH 7.6—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 13 mL of the monobasic potassium phosphate solution with 87 mL of the anhydrous dibasic sodium phosphate solution.

Substrate solution—Dissolve 85.7 mg of N-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying Crystallized Trypsin (see NOTE), in water to make 100 mL. Dilute 10 mL of this solution with *0.067 M Phosphate buffer, pH 7.6* to 100 mL. Determine the absorbance of this solution, in a 1-cm cell, at 253 nm, in a suitable spectrophotometer equipped with thermospacers to maintain a temperature of 25 ± 0.1°, using water as the blank. By the addition of *0.067 M Phosphate buffer, pH 7.6*, or of the *Substrate solution* before dilution, adjust the absorbance so that it measures not less than 0.575 and not more than 0.585. Use this *Substrate solution* within 2 hours.

Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 N hydrochloric acid to obtain a solution containing about 50 to 60 USP Trypsin Units per mL.

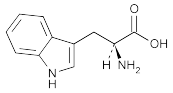
Procedure—Pipet 200 µL of 0.0010 N hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in a spectrophotometer, and adjust the instrument so that the absorbance reads 0.050 at 253 nm. Pipet 200 µL of *Crystallized Trypsin solution*, containing 10 to 12 USP Trypsin Units, into another 1-cm cell, add 3.0 mL of *Substrate solution*, and place the cell in the spectrophotometer. At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for 5 minutes. Repeat the procedure on the same dilution at least once. Plot a curve of absorbance against time, and use only those values that form a straight line to determine the activity of the Crystallized Trypsin. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in this *Assay.*

Calculate the number of USP Trypsin Units per mg taken by the formula:

$$(A_1 - A_2) / (0.003TW)$$

in which A_1 is the absorbance straight-line final reading, A_2 is the absorbance straight-line initial reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbances.

Tryptophan



$C_{11}H_{12}N_2O_2$ 204.23

L-Tryptophan [73-22-3].

DEFINITION

Tryptophan contains NLT 98.5% and NMT 101.5% of $C_{11}H_{12}N_2O_2$, as L-tryptophan, calculated on the dried basis.

IDENTIFICATION

- **INFRARED ABSORPTION** ⟨**197K**⟩

ASSAY

- **PROCEDURE**

Sample solution: Place 200 mg of Tryptophan in a 125-mL flask. Dissolve in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid.

Analysis: Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%
- **CHLORIDE AND SULFATE,** *Chloride* ⟨**221**⟩**:** A 0.73-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.05%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
- **CHLORIDE AND SULFATE,** *Sulfate* ⟨**221**⟩**:** A 0.33-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 N sulfuric acid (0.03%). [NOTE—Gently heat the sample preparation to dissolve, if necessary.]
- **IRON** ⟨**241**⟩**:** NMT 30 ppm
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 15 ppm

Organic Impurities

- **PROCEDURE 1**

Solution A: Trifluoroacetic acid in water (1 mL/L)

Solution B: Trifluoroacetic acid in an acetonitrile and water solution (80:20) (1 mL/L trifluoroacetic acid solution)

Standard solution: 1.0 mg/L each of USP Tryptophan Related Compound A RS and USP Tryptophan Related Compound B RS in water

Sample solution: 10.0 mg/mL of tryptophan in water

System suitability solution: 1.0 mg/L of USP Tryptophan Related Compound B RS in water

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
2	95	5
37	35	65